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SOIL SCIENCE

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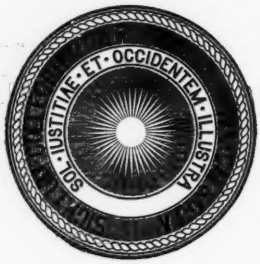

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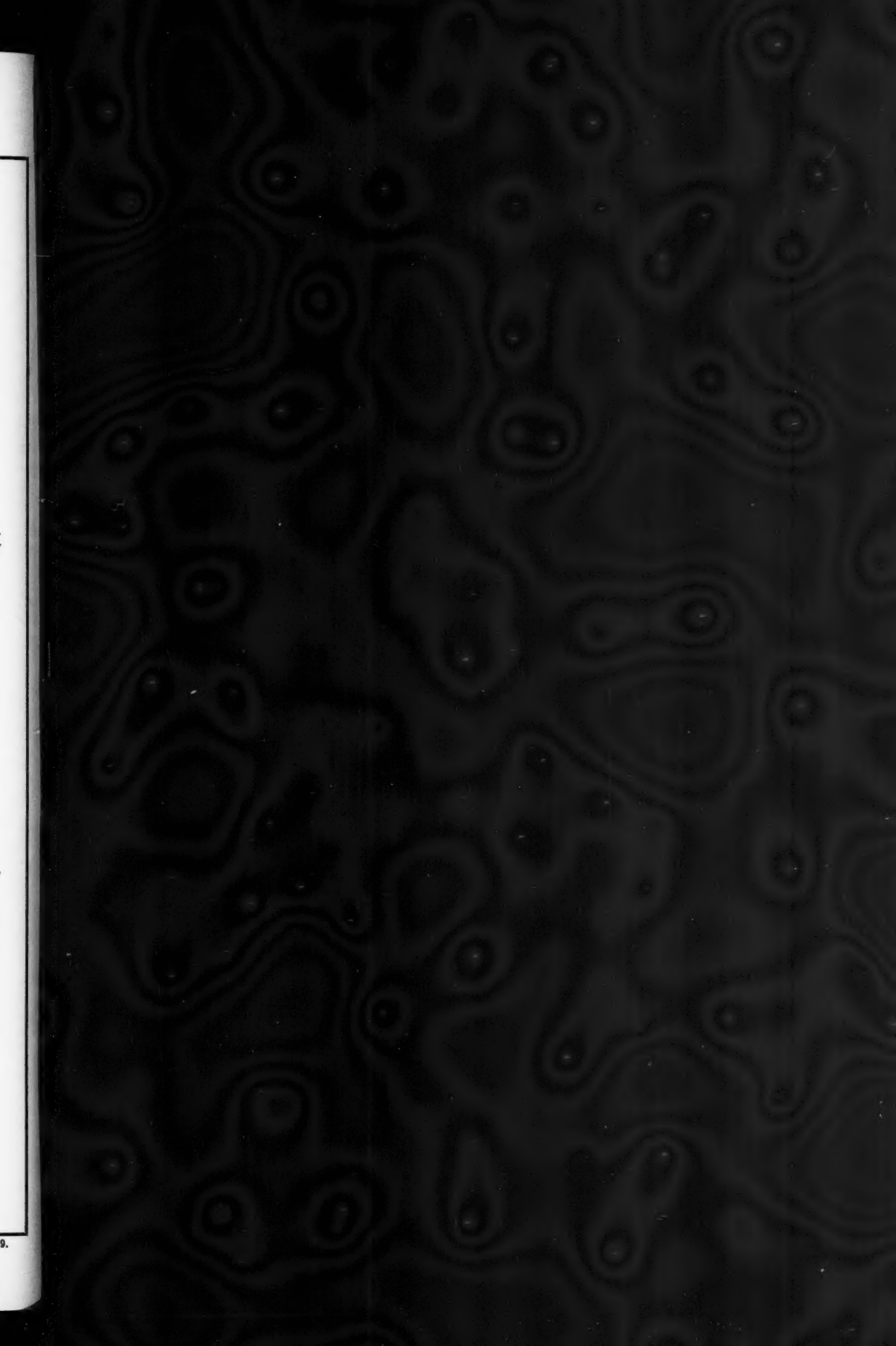

STUDIES ON PROTEIN SYNTHESIS BY THE GENUS AZOTOBACTER

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The biological fixation of atmospheric nitrogen has been the subject of many physiological and biochemical studies, particularly with reference to the metabolism of the nitrogen fixing bacteria. Several theories have been advanced to explain the mechanism of the process of nitrogen fixation, the most plausible of which is that ammonia is the first product of fixation.

The purpose of the present investigation was to determine the products of metabolism of Azotobacter, to compare the biochemical activities of the various species that comprise the genus, and to attempt to explain the mechanism of protein synthesis by these organisms.

EXPERIMENTAL

Four species of the genus Azotobacter were used in this study: *A. chroococcum* Beijerinck, *A. vinelandii* Lipman, *A. agilis* Beijerinck, and *A. beijerinckii* Lipman. Although Bergey (3) includes two additional species in the genus (*A. woodstownii* Lipman and *A. vitreus* Löhnis and Westermann), there is some question whether these species are true Azotobacter. The four species which were used have been the subject of several studies by the author, and have been carefully checked in order to insure that they were typical species.

After a survey of the literature in search of methods which might be used to collect the products of growth for analysis, and after many preliminary tests, the following scheme was adopted: Twenty-four hours prior to the inoculation of plates, the stock cultures were transferred to slants of nitrogen-free mannite agar and incubated for twenty-four hours. At the end of that time, stained preparations were made from each culture and were examined microscopically in order to check the purity of each culture. Contaminating organisms were never detected. Sterile water blanks were then inoculated with each of the cultures used. Several hours prior to this, large plates (200 mm.)

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[2] Contribution from the department of agricultural chemistry and soils. Condensed from a dissertation presented in partial fulfillment of the requirements for the degree of doctor of philosophy, University of Arizona.

of the nitrogen-free agar had been poured and allowed to solidify. The medium had the following composition (8):

Mannitol	10.0 gm.
Dipotassium phosphate (K_2HPO_4)	0.5
Magnesium sulfate ($MgSO_4 \cdot 7\ H_2O$)	0.2
Sodium chloride (NaCl)	0.2
Manganese sulfate ($MnSO_4 \cdot 4\ H_2O$)	Trace
Ferric chloride ($FeCl_3 \cdot 6\ H_2O$)	Trace
Agar	15.0
Distilled water	1,000.0 cc.

When the medium had solidified, 1 cc. of the water suspension was added to the plate, which was then rotated so as to give an even distribution of the suspension upon the surface of the medium. In the latter part of the experiment, large Pyrex trays fitted with copper covers were used in place of the 200-mm. dishes (11). All glassware and media were carefully sterilized, and every care was taken to prevent contamination. The reagents used during the course of the experiment had been previously tested for absence of nitrogen. In each series of plates one culture dish was inoculated with 1 cc. of sterile water to serve as a check. In no case did any colonies develop on these control plates.

The plates were incubated at 30°C., for 4 days. This period was selected because Halversen (12) has shown that at that time 96 per cent of the total nitrogen is in the form of protein, and it is also recognized that older cultures may autolyze, liberating ammonia; also the culture of *A. chroococcum* was an active pigment former, but this pigment did not develop until after the fourth day. Rippel and Ludwig (21) have claimed that the black pigment of this species is due to the conversion of tyrosine to melanin. Consequently, the incubation period was set so that this species might be harvested prior to pigment formation.

At the end of the incubation period, the growth was scraped from the surface of the medium with a glass slide, care being taken not to remove any of the agar. Since the bacterial mass contained from 85 to 95 per cent of water, dehydration was necessary. The investigations of Omeliansky and Sieber (18) had already shown that desiccation in vacuo was not satisfactory; therefore, the bacterial mass was transferred to jars of anhydrous acetone and allowed to stand several days at room temperature, the acetone being changed at frequent intervals. When a sufficient quantity of material had been obtained, the acetone was removed by filtration through hardened filter paper, and the material was air-dried. The residue was then ground in an agate mortar, extracted and dried as before, and finally ground in a ball mill. This method of dehydration had been used successfully by Wilkerson and Gortner (25) in a biochemical study of the pig embryo. Acetone does not denature proteins and does not dissolve fats to any great extent, but does have the disadvantage, particularly when hot, of dissolving phospho-proteins, phospholipids, and similar substances.

The material, which had been obtained in the manner already described, was analyzed. A gross chemical analysis was made according to the methods of the Association of Official Agricultural Chemists (2); a proximate analysis was made, using the method of Waksman and Stevens (24); and finally a protein analysis by Cavett's micromodification (7) of Van Slyke's method. The

TABLE 1

Gross chemical composition of Azotobacter

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Moisture	8.55	9.06	5.61	5.41
Ash	7.55	7.06	4.05	4.00
Protein (N × 6.25)	61.19	56.25	24.68	25.00
Fat	0.62	1.96	0.94	3.25
Carbohydrates (by difference)	22.09	25.67	64.72	62.34
Total	100.00	100.00	100.00	100.00

TABLE 2

The proximate analysis of Azotobacter

(Moisture-free basis)

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Ether-soluble organic matter	0.67	2.14	0.99	3.43
Alcohol-soluble organic matter	1.68	1.26	1.44	1.67
Cold-water-soluble organic matter	8.60	9.54	14.41	10.65
Hot-water-soluble organic matter	15.83	19.03	18.89	14.64
Hemicelluloses	4.60	4.62	0.13	0.13
Celluloses	0.12	0.12	0.13	0.13
Lignin-like material	3.65	3.72	33.18	33.01
Crude protein	50.98	47.22	15.40	17.98
Ash	8.23	7.70	4.29	4.24
Recovery	94.36	95.35	88.86	85.88

results, which appear in tables 1 to 4, are averages of closely agreeing duplicate determinations.

DISCUSSION

The results of the gross chemical analysis (table 1) show that *A. agilis* and *A. vinelandii* contain larger amounts of protein and ash and smaller amounts of carbohydrates than *A. beijerinckii* or *A. chroococcum.*

The proximate analysis (table 2) shows that the four species may be divided into groups: (I) *A. agilis* and *A. vinelandii*; and (II) *A. beijerinckii* and *A.*

chroococcum. The former group is characterized by larger amounts of hemicelluloses, crude protein, and ash; while the latter group has a greater content

TABLE 3

The distribution of nitrogen in the acid hydrolysate of Azotobacter

(Results expressed as percentage of total nitrogen, not corrected for the solubility of the bases)

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Amide N	13.42	20.36	18.25	17.90
Humin N	13.18	15.82	13.67	9.62
Basic N:				
Arginine N	19.19	16.66	20.26	16.25
Histidine N	0.16	0.16	0.17	0.11
Cystine N	0.31	0.29	1.03	0.50
Lysine N	7.24	5.11	4.51	3.14
Filtrate N:				
Amino N	44.33	39.25	40.08	50.34
Non-amino N	1.44	2.25	1.95	2.16
Recovery	99.27	99.90	99.92	100.02
Tyrosine N	1.00	1.22	0.78	0.62
Tryptophane N	0.41	0.19	0.15	0.11

TABLE 4

The distribution of nitrogen in Azotobacter

(Results expressed as percentage of original material, air-dry basis)

	A. AGILIS	A. VINELANDII	A. BEIJERINCKII	A. CHROOCOCCUM
	per cent	*per cent*	*per cent*	*per cent*
Amide N	1.340	1.832	0.720	0.716
Humin N	1.312	1.425	0.540	0.385
Basic N	(2.70)	(2.00)	(1.025)	(0.800)
Arginine N	0.95	0.75	0.40	0.325
Histidine N	0.078	0.073	0.003	0.002
Cystine N	0.034	0.026	0.037	0.020
Lysine N	1.638	1.151	0.585	0.453
Filtrate N:				
Total N	4.55	3.76	1.67	2.10
Amino N	4.41	3.53	1.55	2.01
Non-amino N	0.04	0.23	0.12	0.09
Recovery	9.90	9.00	3.95	4.00
Total N (Kjeldahl method)	9.95	9.00	3.95	4.00

of lignin-like materials. The differences in the amounts of ether-soluble organic matter, alcohol-soluble organic matter, and water-soluble organic matter do not appear to be constant enough to warrant any generalizations.

The Van Slyke analysis did not reveal any constant variations. If, however, the results are expressed in terms of percentage of original material instead of percentage of nitrogen, as in table 4, the four species may be grouped, according to relationships, into the two groups mentioned in the foregoing. Because of the low nitrogen content, these differences are lost in table 3, for if the results are expressed in terms of total nitrogen, the variations are so magnified that the relationships are not apparent.

The results show, then, a decided similarity between *A. agilis* and *A. vinelandii* and between *A. beijerinckii* and *A. chroococcum*. This is in accord with the findings of Löhnis and Smith (17), who concluded from a study of cultural characteristics and life histories of many strains of Azotobacter that "only two species of Azotobacter are characterized thus far: *Azotobacter chroococcum* and *A. agile* Beij. (syn. *A. vinelandii* J. G. Lipman). *A. beijerinckii* Lipman is a variety of *A. chroococcum*, and *A. vitreum* Löhnis is probably a variety of *A. agile*. *A. smyrnii* C. B. Lipman et Burgess can not be accepted as a species; according to all marks ascribed to it by its authors, it is a large sporulating growth type of *A. chroococcum*. *A. hilgardii* C. B. Lipman and *A. woodstownii* J. G. Lipman are both incompletely described and should not be retained." Aso and Yoshida (1), by means of serum reactions (compliment fixation), have been able to distinguish various types of Azotobacter. Their results show that Azotobacter can be classified into three types: *A. chroococcum*, *A. vinelandii*, and *A. vitreus*. They found that *A. chroococcum* and *A. beijerinckii* are of the same type and that apparently the latter is a strain of the former. The author has shown elsewhere (10) that over a wide range of temperatures *A. agilis* and *A. vinelandii* were more active nitrogen fixers than *A. beijerinckii* or *A. chroococcum*. The ratio of nitrogen fixed is not proportional to the nitrogen content of the particular species, however, which would indicate that since *A. chroococcum* and *A. beijerinckii* have lower nitrogen content, they must have a higher growth rate. Many soil bacteriologists have considered *A. chroococcum* to be the most active species of the genus, but this is probably because few studies have been made in which all the species were compared, and also because *A. chroococcum* is more widely distributed than the other species

The results show, therefore, that *A. agilis* and *A. vinelandii* are closely related, and that a similar relationship exists between *A. chroococcum* and *A. beijerinckii*.

Larmour (16) has compiled the results of the analyses of many proteins. When the results of the Van Slyke determination are compared with those given by Larmour, the values for humin nitrogen are much higher than any he cites, and the values for amide nitrogen are also quite high. It is probable that the carbohydrate fraction may be partially responsible for these results. Hydrolysis with hydrochloric acid may convert a portion of the carbohydrates to aldehydes, and a subsequent condensation with tryptophane would form humin (9). The high values for ammonia may be due either to deaminization of amino acids during hydrolysis or to the presence of free ammonia resulting

from the fixation of atmospheric nitrogen, and which had not been converted into amino acids. It may be, however, that the nitrogen fixing organisms are characterized by the presence of large amounts of amino nitrogen. In a study of the proteins of the nodules of *Vicia faba*, Paresi and Masetti-Zannini (19) report the following results: ammonia nitrogen, 18.80 per cent; humin nitrogen, 12.53 per cent; basic nitrogen, 16.91 per cent; non-basic nitrogen, 50.87 per cent (recovery 99.11 per cent).

Arginine and lysine are the principal amino acids of the basic fraction. Small amounts of cystine and histidine are also found. The non-basic fraction contains the larger part of the amino acids, and, unfortunately, satisfactory methods are not available for determining them. These amino acids are probably glycine, alanine, valine, phenylalinin, and other amino acids.

The tyrosine content of these organisms is very small, and it is interesting to note that *A. chroococcum* contains the smallest amount of the four species. Rippel and Ludwig (21) have claimed that the black pigment formed by *A. chroococcum* is due to the conversion of tyrosine and melanin. The stock culture of *A. chroococcum* was selected because of the intense pigment that it formed. Ranganathan and Norris (20) were not able, however, to demonstrate the presence of tyrosinase in a culture of *A. chroococcum*. If pigmentation of this species is due to the conversion of tyrosine to melanin, it is difficult to explain why the other species do not form pigment so readily under identical conditions, since they all contain at least as much tyrosine as does *A. chroococcum*, unless the enzyme tryrosinase is found only in *A. chroococcum*. This is particularly true in the case of *A. beijerinckii*, which appears to be very closely related morphologically, culturally, serologically, and chemically to *A. chroococcum*.

The results given in the Van Slyke analysis do not agree well with those given by Omeliansky and Sieber (18) for *A. chroococcum*. These investigators used a different agar and a longer incubation period. These factors, together with a different method of obtaining material and subsequent modifications of analytical methods, may account for the differences. The author realized at the beginning of this investigation that there are many objections and limitations to the Van Slyke method, but it appeared to be the best method available.

In general, the beijerinckii-chroococcum group contained larger amounts of water soluble organic matter than the agilis-vinelandii group. This consisted chiefly of protein and materials which gave a positive test with Molisch's reagent but which did not reduce Fehling's solution or give a positive test for starch. This carbohydrate fraction may be the gum which Hamilton (13) has described for *A. chroococcum*. Further tests were not made upon this fraction.

Within recent years, studies upon respiration have shown that substances which may act as hydrogen acceptors or donors exist within animal and bacterial cells. In the animal body, glutathione plays an important part in the oxidation-reduction system. Callow and Robinson (6) have reported the

presence of glutathione in many species of bacteria, and although their results have been questioned, there exists in bacteria some substance capable of giving a positive test with sodium nitroprusside. This test has been considered specific for the sulfydryl (—SH) group, but it is now recognized that other substances may give a positive test with this reagent.

In order to determine whether Azotobacter contained any substance capable of acting as a hydrogen accepter or donor, the material which had been used in the other determinations was tested for glutathione, by the method of Callow and Robinson (6). Each species of Azotobacter gave a positive reaction. Although this test alone is not sufficient evidence to verify the presence of glutathione in Azotobacter, the conclusion does not seem improbable, and possibly a part of the cystine found in the Van Slyke analysis might result from the oxidation of cysteine, which is a part of the glutathione molecule.

The mechanism of protein synthesis by Azotobacter

Space will not permit an extensive discussion of the mechanism of protein synthesis, especially since such a discussion must be hypothetical. It is now generally recognized that the first product of nitrogen fixation by Azotobacter is ammonia. The ammonia is then converted into amino acids by combining with various organic acids: with acetic acid, glycine is produced; with propionic acid, alanine is produced; with phenyl-hydroxy-propionic acid, tyrosine results; and in a similar way other amino acids might be formed.

The nitrogen fixed by these organisms comes from the atmosphere; hydrogen cannot, since it occurs in the air in extremely small amounts. Hydrogen is therefore obtained during the breakdown of carbohydrates. In this case, Kluyver's (15) theories of respiration and photosynthesis, involving hydrogenations and dehydrogenations, are applicable and may be represented by the following equation:

$$X \text{ (Catalysts)} + H_2A = XH_2 + A$$
(A may be O, S, or nothing)

In the case of nitrogen fixation, the equation would be:

$$X + N_2 = 2XN$$
$$XN + H_2A = XNH_2 + A$$
$$XNH_2 + 2H = NH_3 + X$$

This is essentially the same mechanism as that suggested by Blom (4). He assumes that organic iron compounds are the catalysts.

Recent studies in carbohydrate metabolism, both animal and microbiological, have suggested a mechanism of carbohydrate breakdown which is very similar. Space will not permit a discussion of these theories, which are fully discussed by Buchanan and Fulmer (5), Hardin (14), Kluyver (15), and Stephenson (22). In general, the sugar molecule (hexose) is converted into methyl-glyoxal (or some activated form of it) and into pyruvic acid. From this stage, it may

be converted by hydrogenations and dehydrogenations into various acids; the carbon chain may be shortened by decarboxylation or lengthened by condensations.

This postulated mechanism of protein synthesis may be summarized as follows: In the process of carbohydrate breakdown, various organic acids are formed, and the active hydrogen produced in the processes is utilized by the organisms for the fixation of nitrogen (probably a direct union to form NH_3). Ammonia reacts with the respective organic acids to form amino acids, which are subsequently converted into protein.

Table 2 shows that Azotobacter are able to synthesize rather complex carbohydrate materials. There may be a relationship between protein synthesis and gum production by these organisms. Hamilton (13) has shown that the amount of gum produced by *A. chroococcum* was not greatly affected by simple carbohydrates, but that polysaccharides led to increased gum production. Gum is not formed in peptone solutions. Since this gum does not contain nitrogen, its production may be associated with protein synthesis, probably in the production of active hydrogen for fixation. The decomposition of carbohydrates liberates energy for these organisms. If, however, small amounts of nitrogen are fixed, as a result of the utilization of nitrogen compounds, small quantities of activated hydrogen are required, which means low respiration and small amounts of available energy. Gum formation by Azotobacter may be considered as a sort of "luxury synthesis." This may account for the difference in hemicellulose content of these organisms. *A. agilis* and *A. vinelandii* fixed larger amounts of nitrogen, hence more energy was available and larger amounts of hemicellulose were synthesized. The energy produced from carbohydrates in excess of growth requirements is probably used in the production of complex carbohydrates.

SUMMARY

Four species of Azotobacter were grown on nitrogen-free mannitol agar, and the bacterial growth was analyzed.

A. vinelandii and *A. agilis* are very similar in composition; a close similarity exists between *A. chroococcum* and *A. beijerinckii*. This relationship also exists in their nitrogen fixing abilities. A Van Slyke distribution did not reveal consistently wide differences but did indicate this similarity.

Arginine and lysine were the amino acids found in largest amounts. Tyrosine, tryptophane, cystine, and histidine were found in smaller amounts. Approximately 40 per cent of the total nitrogen was found in the non-basic fraction, which indicates the presence of simpler amino acids (glycine, alanine, etc.).

Qualitative tests showed the presence of a substance giving a positive reaction with sodium nitroprusside. This substance may be glutathione.

Semi-quantitative determinations indicated that the proteins present were chiefly globulins, glutelins, and albumins.

The metabolism of Azotobacter and the formation of amino acids are discussed briefly.

REFERENCES

(1) Aso, K., and Yoshida, R. 1928 Application of the serum reaction in the classification of Azotobacter. *Proc. and Papers Internat. Cong. Soil Sci.* 3 (Comn. 3 and 4): 150–151.

(2) Association of Official Agricultural Chemists 1931 Methods of Analysis. Washington, D. C.

(3) Bergey, D. H. 1930 Manual of Determinative Bacteriology. The Williams & Wilkins Co., Baltimore.

(4) Blom, J. 1931 Ein Versuch, die chemischen Vorgange bei der Assimilation des molekularen Stickstoffs durch Mikroorganismens zu erklaren. *Centbl. Bakt.* (II) 84: 60–86.

(5) Buchanan, R. E., and Fulmer, E. I. 1928, 1930 Physiology and Biochemistry of Bacteria, vol. I and III. The Williams & Wilkins Co., Baltimore.

(6) Callow, A. B., and Robinson, M. E. 1925 The nitroprusside reaction of bacteria. *Biochem. Jour.* 19: 19–24.

(7) Cavett, J. W. 1932 A modification of the Van Slykes nitrogen distribution method. *Jour. Biol. Chem.* 95: 335–343.

(8) Fred, E. B., and Waksman, S. A. 1928 Laboratory Manual of General Microbiology. McGraw-Hill Book Co., New York.

(9) Gortner, R. A. 1929 Outline of Biochemistry. John Wiley and Sons, New York.

(10) Greene, R. A. 1932 The effect of temperature upon nitrogen fixation by Azotobacter. *Soil Sci.* 33: 153–161.

(11) Greene, R. A. 1935 A mass culture apparatus for securing bacterial cells for analysis. *Jour. Lab. and Clin. Med.* (Accepted for publication.)

(12) Halversen, W. V. 1927 The nitrogen metabolism of nitrogen-fixing bacteria. *Iowa State Col. Jour. Sci.* 1: 395–411.

(13) Hamilton, W. B. 1931 Gum production by Azotobacter Chroococcum of Beijerinck and its composition. *Jour. Bact.* 22: 249–254.

(14) Harden, A. 1923 Alcoholic Fermentation, ed. 3. Longmans, Green & Co., New York.

(15) Kluyver, A. J. 1931 The chemical activities of microorganisms. University of London Press, London.

(16) Larmour, R. K. 1928 Correlations between the total nitrogen of the bases and arginine nitrogen and between the total nitrogen of the bases and lysine nitrogen, of various proteins. *Trans. Royal Soc. Canada. 3rd Ser.* 22: 349–363.

(17) Löhnis, F., and Smith, N. R. 1923 Studies upon the life cycles of the bacteria. *Jour. Agr. Res.* 23: 401–432.

(18) Omeliansky, W. L., and Sieber, N. O. 1913 Zur Frage nach der chemischen Zusammemsetzung der Bakterienkorper des Azotobacter chroococcum. *Ztschr. Physiol. Chem.* 88: 445–459.

(19) Parisi, E., and Masetti-Zannini, C. 1926 Le sostanze proteiche dei tubercoli delle leguminose. *Staz. Sper. Agr. Ital.* 59: 207–228.

(20) Ranganathan, S., and Norris, R. V. 1927 Nitrogen fixation by Azotobacter chroococcum. *Jour. Indian. Inst. Sci.* 10A (part 8): 79–96.

(21) Rippel, A., and Ludwig, O. 1925 The darkening of Azotobacter chroococcum Beij. by melanin formation. *Centbl. Bakt.* (II) 64: 161–166 (Abs. in *Chem. Abs.* 19: 2684).

(15), Dean (1), Ford (4), Fraps (5), Fudge (6), Gaarder (7), and Heck (10) have been reviewed.

To summarize the work and opinions of these ten workers, it may be said that all of them believe that pH and degree of saturation of the soil with PO_4 are of greatest importance in all soils. Most of them consider that hydrated oxides of Fe and Al are the chief causes of fixation in non-alkaline soils. Four find that easily soluble silicates increase the availability of PO_4. Although easily soluble Ca and Mg in soil quickly fix soluble phosphates, the insoluble compounds formed are fairly available to plants in non-alkaline soils. But McGeorge and Breazeale find that in calcareous soils, basic very insoluble and non-available phosphates are formed. Demolon considers that anion exchange is the cause of much of the increased solubility of PO_4 produced by addition of soluble silicates, and this view is in accord with the point made by Clarens, and quoted by Thomas, that the clay molecule contains a basic OH group replaceable by acid radicals, such as PO_4. Most of them agree that organic matter, especially soluble organic matter, increases the solubility and penetrating power of soil phosphate. Also, organic compounds of P are much less fixed than inorganic. McGeorge and Breazeale, the only ones who worked much with calcareous soils, think that the unavailability of PO_4 in these soils is due not only to high pH in which phosphates of Ca and Mg are insoluble but still more to formation of very insoluble basic carbonate-apatite.[2]

EXPERIMENTAL

The general character of the soils used in the experiments, with respect to phosphate supplying power, is presented in table 1.

The subject studied was under the topics:

1. Measurement
2. Phenomena
3. Causes
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} of fixation

Measurement

Some means of measuring the fixing power of a soil seems necessary to an intelligent study of the subject.

A review of the procedures suggested by Heck (22, p. 20), Lohse (12), McCool (14), Gile (9), Ford (4), Fraps (5), and Fudge (6) reveals that all of them are empirical, and that no one method has been generally or officially accepted. What is wanted is a method that will give reliable and reproducible results with least expense of time, labor, equipment, and money. The one adopted herein, chiefly on account of convenience and rapidity, consists in adding a soluble phosphate to a mixture of soil and water, agitating for one hours, filtering, and in the filtrate determining the phosphate by the molyb-

[2] A more extended review of the papers of these investigators has been omitted in order to save space.

denum-blue method. Two variations have been used. The same amount of phosphate is added to all soils, in which case the PO_4 in the extract may be extremely variable, or a variable amount is added, according to the fixing power of the soil, so that the extract will always have about the same concentration of PO_4, e.g. 1 p.p.m.

Soils differ greatly in fixing power; consequently, in using the first method, unless a relatively large amount of PO_4 is added to all soils, the fixing power of high fixing soils will not be represented in proper proportion to that of low fixing soils. This is shown in table 2 with soils 59 and 35, both of which fix 99+ per cent of added PO_4, though the highest addition to soil 59 contains sixteen times as much PO_4 as the lowest. On the other hand, if enough PO_4 is added so that the extract will contain 1 p.p.m., or any other arbitrary concentration, the amounts thus required will much better indicate the relative

TABLE 1

General character of soils used in fixation experiments

SOIL NO. AND NAME	FIXING POWER	pH	PO_4		
			Truog	Water extract	
			p.p.m. in soil	1:1 p.p.m. in solution	1:100 p.p.m. in solution
1c. Yolo silty clay loam	40	6.7	620	0.20	0.18
30. Fresno fine sandy loam	0	6.9	440	5.00	0.40
35. Holland loam	170	5.4	20	0.04	0.03
37. Nord sandy loam	...	8.2	400	0.58	0.18
38. Vina clay loam	80	7.2	248	0.18	0.21
53. Delhi sand	0	7.1	88	1.32	0.27
59. Aiken clay	460	6.0	4	0	0
64. Vina silt loam	70	6.9	160	0.10	0.08
78. Gold Ridge sandy loam	35	4.8	20	0.20	0.06
95. Ramona loam	7	8.3	144	0.80	0.24

fixing power of different soils. In other words, *if the amount necessary to give the desired concentration in the extract is taken as a measure of fixing power*, instead of the amount actually fixed, a more reliable estimate of the character of the soil is obtained. In figure 1, the graphs plotted from table 2 for soils 59 and 35 show that an increasing proportion of the PO_4 added remains in solution as the amount added increases, indicating that no definite compound is formed. Gile (9) observed the same thing.

In practice, the second method usually requires more than one trial to find the amount needed, so that three 100-gm. portions of air-dry soil are mixed with three 100-cc. portions of water containing three quite different amounts of PO_4. The mixtures, placed in 300-cc. bottles, are shaken occasionally for one hour, then filtered, and the PO_4 in the filtrate is determined. From the three figures found may be calculated the amount that must be added in order

to obtain an extract containing 1 p.p.m. PO_4. This amount in milligrams per kilogram of soil, is called the "fixing power" of the soil. Some soils may contain that or a greater amount without any added PO_4. These soils, as well as those to which it has been added, may still be able to fix large quantities of PO_4.

TABLE 2

Fixation of PO_4 by soils from different amounts added to 100 gm. soil

SOIL NO.	PO_4 ADDED	PO_4 REMAINING IN SOLUTION	PER CENT FIXED OF AMOUNT ADDED	FIXING POWER—PO_4 PER KGM. OF SOIL
	mgm.	*p.p.m.*		*mgm.*
59	5	0.06	99.9	
	10	0.12	99.9	
	20	0.18	99.9	
	40	0.80	99.9	460
	50	1.16	99.8	
	60	3.20	99.5	
	80	3.34	99.6	
64	5	0.66	98.7	
	7	1.00	98.6	70
	10	1.44	98.6	
38	5	0.48	99.0	
	8	1.00	98.7	80
	10	1.14	98.8	
78	3	0.72	97.6	35
	4	1.24	96.9	
	5	1.34	97.3	
	10	4.00	96.0	
35	5	0.16	99.7	
	10	0.32	99.7	
	15	0.64	99.5	170
	20	1.76	99.1	
	40	8.00	98.0	
1c	3	0.88	97.1	
	4	1.00	97.5	40
	5	1.60	96.8	
	10	2.50	97.5	

In order to make the determination of fixing power more similar to the effect of adding phosphate to soil in the field, 100 gm. of soil was placed in a 48 mm. diameter percolator tube and, after addition of PO_4, was leached with water or other solvent. Slight differences in preparation of the setup make such great differences in the rate of percolation and in the concentration of PO_4

in the percolate, that the results are, in most cases, not reproducible. When the phosphate was applied in a solution of 100 p.p.m. concentration, the PO_4 appeared in the percolate after most of the fixing power of the soil had been satisfied. This may require several times as much PO_4 as in an equilibrium

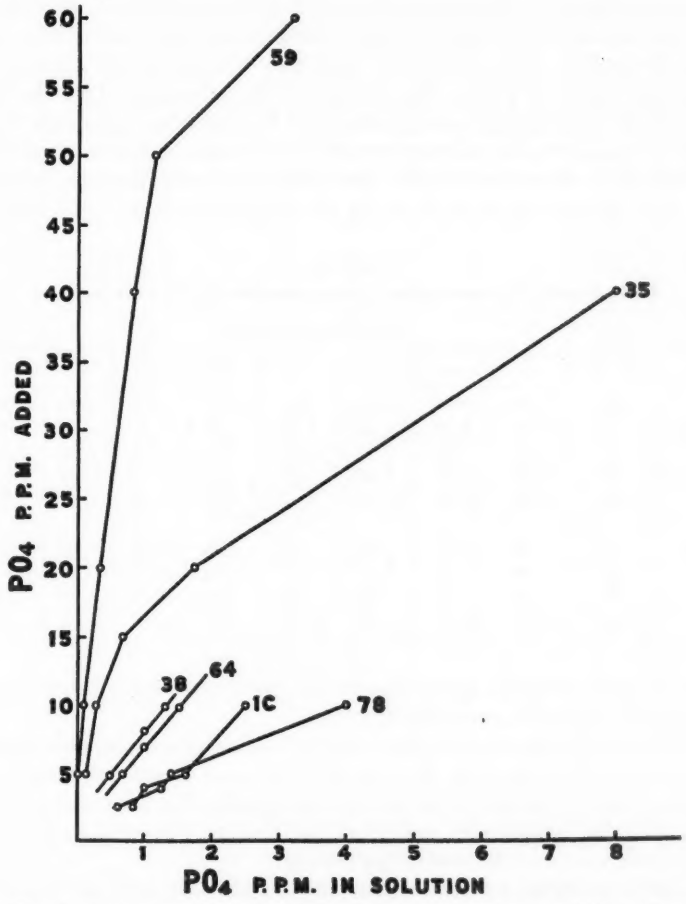

FIG. 1. FIXATION OF PO_4 FROM DIFFERENT AMOUNTS ADDED TO 100 GM. SOIL

extract and may be a very tedious procedure. The first 100 or 200 cc. of percolate may contain practically no PO_4. In table 3 are recorded the results obtained on a few soils by this method. It is so difficult to control and to produce duplicate results that this percolation method is considered very unsatisfactory for measuring fixing power. When the phosphate was applied as a dry

be converted by hydrogenations and dehydrogenations into various acids; the carbon chain may be shortened by decarboxylation or lengthened by condensations.

This postulated mechanism of protein synthesis may be summarized as follows: In the process of carbohydrate breakdown, various organic acids are formed, and the active hydrogen produced in the processes is utilized by the organisms for the fixation of nitrogen (probably a direct union to form NH_3). Ammonia reacts with the respective organic acids to form amino acids, which are subsequently converted into protein.

Table 2 shows that Azotobacter are able to synthesize rather complex carbohydrate materials. There may be a relationship between protein synthesis and gum production by these organisms. Hamilton (13) has shown that the amount of gum produced by *A. chroococcum* was not greatly affected by simple carbohydrates, but that polysaccharides led to increased gum production. Gum is not formed in peptone solutions. Since this gum does not contain nitrogen, its production may be associated with protein synthesis, probably in the production of active hydrogen for fixation. The decomposition of carbohydrates liberates energy for these organisms. If, however, small amounts of nitrogen are fixed, as a result of the utilization of nitrogen compounds, small quantities of activated hydrogen are required, which means low respiration and small amounts of available energy. Gum formation by Azotobacter may be considered as a sort of "luxury synthesis." This may account for the difference in hemicellulose content of these organisms. *A. agilis* and *A. vinelandii* fixed larger amounts of nitrogen, hence more energy was available and larger amounts of hemicellulose were synthesized. The energy produced from carbohydrates in excess of growth requirements is probably used in the production of complex carbohydrates.

SUMMARY

Four species of Azotobacter were grown on nitrogen-free mannitol agar, and the bacterial growth was analyzed.

A. vinelandii and *A. agilis* are very similar in composition; a close similarity exists between *A. chroococcum* and *A. beijerinckii*. This relationship also exists in their nitrogen fixing abilities. A Van Slyke distribution did not reveal consistently wide differences but did indicate this similarity.

Arginine and lysine were the amino acids found in largest amounts. Tyrosine, tryptophane, cystine, and histidine were found in smaller amounts. Approximately 40 per cent of the total nitrogen was found in the non-basic fraction, which indicates the presence of simpler amino acids (glycine, alanine, etc.).

Qualitative tests showed the presence of a substance giving a positive reaction with sodium nitroprusside. This substance may be glutathione.

Semi-quantitative determinations indicated that the proteins present were chiefly globulins, glutelins, and albumins.

The metabolism of Azotobacter and the formation of amino acids are discussed briefly.

REFERENCES

(1) Aso, K., and Yoshida, R. 1928 Application of the serum reaction in the classification of Azotobacter. *Proc. and Papers Internat. Cong. Soil Sci.* 3 (Comn. 3 and 4): 150–151.

(2) Association of Official Agricultural Chemists 1931 Methods of Analysis. Washington, D. C.

(3) Bergey, D. H. 1930 Manual of Determinative Bacteriology. The Williams & Wilkins Co., Baltimore.

(4) Blom, J. 1931 Ein Versuch, die chemischen Vorgange bei der Assimilation des molekularen Stickstoffs durch Mikroorganismens zu erklaren. *Centbl. Bakt.* (II) 84: 60–86.

(5) Buchanan, R. E., and Fulmer, E. I. 1928, 1930 Physiology and Biochemistry of Bacteria, vol. I and III. The Williams & Wilkins Co., Baltimore.

(6) Callow, A. B., and Robinson, M. E. 1925 The nitroprusside reaction of bacteria. *Biochem. Jour.* 19: 19–24.

(7) Cavett, J. W. 1932 A modification of the Van Slykes nitrogen distribution method. *Jour. Biol. Chem.* 95: 335–343.

(8) Fred, E. B., and Waksman, S. A. 1928 Laboratory Manual of General Microbiology. McGraw-Hill Book Co., New York.

(9) Gortner, R. A. 1929 Outline of Biochemistry. John Wiley and Sons, New York.

(10) Greene, R. A. 1932 The effect of temperature upon nitrogen fixation by Azotobacter. *Soil Sci.* 33: 153–161.

(11) Greene, R. A. 1935 A mass culture apparatus for securing bacterial cells for analysis. *Jour. Lab. and Clin. Med.* (Accepted for publication.)

(12) Halversen, W. V. 1927 The nitrogen metabolism of nitrogen-fixing bacteria. *Iowa State Col. Jour. Sci.* 1: 395–411.

(13) Hamilton, W. B. 1931 Gum production by Azotobacter Chroococcum of Beijerinck and its composition. *Jour. Bact.* 22: 249–254.

(14) Harden, A. 1923 Alcoholic Fermentation, ed. 3. Longmans, Green & Co., New York.

(15) Kluyver, A. J. 1931 The chemical activities of microorganisms. University of London Press, London.

(16) Larmour, R. K. 1928 Correlations between the total nitrogen of the bases and arginine nitrogen and between the total nitrogen of the bases and lysine nitrogen, of various proteins. *Trans. Royal Soc. Canada. 3rd Ser.* 22: 349–363.

(17) Löhnis, F., and Smith, N. R. 1923 Studies upon the life cycles of the bacteria. *Jour. Agr. Res.* 23: 401–432.

(18) Omeliansky, W. L., and Sieber, N. O. 1913 Zur Frage nach der chemischen Zusammemsetzung der Bakterienkorper des Azotobacter chroococcum. *Ztschr. Physiol. Chem.* 88: 445–459.

(19) Parisi, E., and Masetti-Zannini, C. 1926 Le sostanze proteiche dei tubercoli delle leguminose. *Staz. Sper. Agr. Ital.* 59: 207–228.

(20) Ranganathan, S., and Norris, R. V. 1927 Nitrogen fixation by Azotobacter chroococcum. *Jour. Indian. Inst. Sci.* 10A (part 8): 79–96.

(21) Rippel, A., and Ludwig, O. 1925 The darkening of Azotobacter chroococcum Beij. by melanin formation. *Centbl. Bakt.* (II) 64: 161–166 (Abs. in *Chem. Abs.* 19: 2684).

(22) STEPHENSON, M. 1930 Bacterial Metabolism. Longmans, Green & Co., London.
(23) WAKSMAN, S. A. 1932 Principles of Soil Microbiology. Williams & Wilkins Co., Baltimore.
(24) WAKSMAN, S. A., AND STEVENS, K. R. 1928 Contribution to the chemical composition of peat: I. Chemical nature of organic complexes in peat and methods of analysis. *Soil Sci.* 26: 113–137.
(25) WILKERSON, V. A., AND GORTNER, R. A. 1932 The chemistry of embryonic growth. III. A biochemical study of the embryonic growth of the pig with special reference to the nitrogenous compounds. *Amer. Jour. Physiol.* 102: 153–166.

FACTORS INFLUENCING PHOSPHATE FIXATION IN SOILS

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Though phosphate fixation in soils has been studied ever since Way, in 1850, discovered its existence, yet our knowledge of it is still fragmentary. The present paper is a report of the study of some of the factors concerned in fixation of phosphate, with some discussion. Knowledge of fixation is necessary to intelligent use of fertilizers with respect to amount, kind, and most effective placement of the phosphate.

In this paper, the term "fixation" designates the removal of PO_4 from a water solution by the soil. Numerous other materials also have the power of fixation. When PO_4[1] has been fixed by soil or other material, it is no longer easily soluble in water and may not be easily soluble in such relatively weak solvents as are commonly used to measure the availability of soil phosphate.

Ford (4, p. 6) says "Fixation is the term applied to the phenomenon whereby soluble phosphorus is removed from solution by soils. It is often designated by other terms such as 'absorption,' 'adsorption,' or 'reversion'."

The work here reported was done for the purpose of obtaining more detailed and exact information on the subject of fixation by means of chemical laboratory experiments. It was sought to learn how fixation is influenced by a variety of treatments mostly confined to the effects of water alone or with small amounts of added salts, such as may occur in the soil solution. Fixation caused by iron or aluminum compounds as such was very little studied. This has been fairly well cleared up by the studies of Gile (9), Weiser (22), Ford (4), Heck (10), Gaarder (7), Dean (1), and others.

Some of the experiments were not carried far enough or were not repeated sufficiently to furnish a basis for entirely valid conclusions. But the results obtained are suggestive of further experimental work which might reveal facts of much importance. The writer may not continue the work, therefore, he offers this report as suggestive rather than conclusive.

No complete search of the literature has been made in connection with this study. Some of the recent papers have been examined, but most of the experimental work was done before some of the important recent papers were in print.

The papers of Gile (9), Demolon (2), Thomas (21), McGeorge and Breazeale

[1] In this paper the expression "PO_4" is meant to include any one or all of the phosphate ions, PO_4^{---}, HPO_4^{--}, or $H_2PO_4^{-}$.

(15), Dean (1), Ford (4), Fraps (5), Fudge (6), Gaarder (7), and Heck (10) have been reviewed.

To summarize the work and opinions of these ten workers, it may be said that all of them believe that pH and degree of saturation of the soil with PO_4 are of greatest importance in all soils. Most of them consider that hydrated oxides of Fe and Al are the chief causes of fixation in non-alkaline soils. Four find that easily soluble silicates increase the availability of PO_4. Although easily soluble Ca and Mg in soil quickly fix soluble phosphates, the insoluble compounds formed are fairly available to plants in non-alkaline soils. But McGeorge and Breazeale find that in calcareous soils, basic very insoluble and non-available phosphates are formed. Demolon considers that anion exchange is the cause of much of the increased solubility of PO_4 produced by addition of soluble silicates, and this view is in accord with the point made by Clarens, and quoted by Thomas, that the clay molecule contains a basic OH group replaceable by acid radicals, such as PO_4. Most of them agree that organic matter, especially soluble organic matter, increases the solubility and penetrating power of soil phosphate. Also, organic compounds of P are much less fixed than inorganic. McGeorge and Breazeale, the only ones who worked much with calcareous soils, think that the unavailability of PO_4 in these soils is due not only to high pH in which phosphates of Ca and Mg are insoluble but still more to formation of very insoluble basic carbonate-apatite.[2]

EXPERIMENTAL

The general character of the soils used in the experiments, with respect to phosphate supplying power, is presented in table 1.

The subject studied was under the topics:

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Measurement

Some means of measuring the fixing power of a soil seems necessary to an intelligent study of the subject.

A review of the procedures suggested by Heck (22, p. 20), Lohse (12), McCool (14), Gile (9), Ford (4), Fraps (5), and Fudge (6) reveals that all of them are empirical, and that no one method has been generally or officially accepted. What is wanted is a method that will give reliable and reproducible results with least expense of time, labor, equipment, and money. The one adopted herein, chiefly on account of convenience and rapidity, consists in adding a soluble phosphate to a mixture of soil and water, agitating for one hours, filtering, and in the filtrate determining the phosphate by the molyb-

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denum-blue method. Two variations have been used. The same amount of phosphate is added to all soils, in which case the PO_4 in the extract may be extremely variable, or a variable amount is added, according to the fixing power of the soil, so that the extract will always have about the same concentration of PO_4, e.g. 1 p.p.m.

Soils differ greatly in fixing power; consequently, in using the first method, unless a relatively large amount of PO_4 is added to all soils, the fixing power of high fixing soils will not be represented in proper proportion to that of low fixing soils. This is shown in table 2 with soils 59 and 35, both of which fix 99+ per cent of added PO_4, though the highest addition to soil 59 contains sixteen times as much PO_4 as the lowest. On the other hand, if enough PO_4 is added so that the extract will contain 1 p.p.m., or any other arbitrary concentration, the amounts thus required will much better indicate the relative

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1c. Yolo silty clay loam	40	6.7	620	0.20	0.18
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64. Vina silt loam	70	6.9	160	0.10	0.08
78. Gold Ridge sandy loam	35	4.8	20	0.20	0.06
95. Ramona loam	7	8.3	144	0.80	0.24

fixing power of different soils. In other words, *if the amount necessary to give the desired concentration in the extract is taken as a measure of fixing power,* instead of the amount actually fixed, a more reliable estimate of the character of the soil is obtained. In figure 1, the graphs plotted from table 2 for soils 59 and 35 show that an increasing proportion of the PO_4 added remains in solution as the amount added increases, indicating that no definite compound is formed. Gile (9) observed the same thing.

In practice, the second method usually requires more than one trial to find the amount needed, so that three 100-gm. portions of air-dry soil are mixed with three 100-cc. portions of water containing three quite different amounts of PO_4. The mixtures, placed in 300-cc. bottles, are shaken occasionally for one hour, then filtered, and the PO_4 in the filtrate is determined. From the three figures found may be calculated the amount that must be added in order

to obtain an extract containing 1 p.p.m. PO_4. This amount in milligrams per kilogram of soil, is called the "fixing power" of the soil. Some soils may contain that or a greater amount without any added PO_4. These soils, as well as those to which it has been added, may still be able to fix large quantities of PO_4.

TABLE 2

Fixation of PO_4 by soils from different amounts added to 100 gm. soil

SOIL NO.	PO_4 ADDED	PO_4 REMAINING IN SOLUTION	PER CENT FIXED OF AMOUNT ADDED	FIXING POWER—PO_4 PER KGM. OF SOIL
	mgm.	*p.p.m.*		*mgm.*
59	5	0.06	99.9	
	10	0.12	99.9	
	20	0.18	99.9	
	40	0.80	99.9	460
	50	1.16	99.8	
	60	3.20	99.5	
	80	3.34	99.6	
64	5	0.66	98.7	
	7	1.00	98.6	70
	10	1.44	98.6	
38	5	0.48	99.0	
	8	1.00	98.7	80
	10	1.14	98.8	
78	3	0.72	97.6	35
	4	1.24	96.9	
	5	1.34	97.3	
	10	4.00	96.0	
35	5	0.16	99.7	
	10	0.32	99.7	
	15	0.64	99.5	170
	20	1.76	99.1	
	40	8.00	98.0	
1c	3	0.88	97.1	
	4	1.00	97.5	40
	5	1.60	96.8	
	10	2.50	97.5	

In order to make the determination of fixing power more similar to the effect of adding phosphate to soil in the field, 100 gm. of soil was placed in a 48 mm. diameter percolator tube and, after addition of PO_4, was leached with water or other solvent. Slight differences in preparation of the setup make such great differences in the rate of percolation and in the concentration of PO_4

in the percolate, that the results are, in most cases, not reproducible. When the phosphate was applied in a solution of 100 p.p.m. concentration, the PO_4 appeared in the percolate after most of the fixing power of the soil had been satisfied. This may require several times as much PO_4 as in an equilibrium

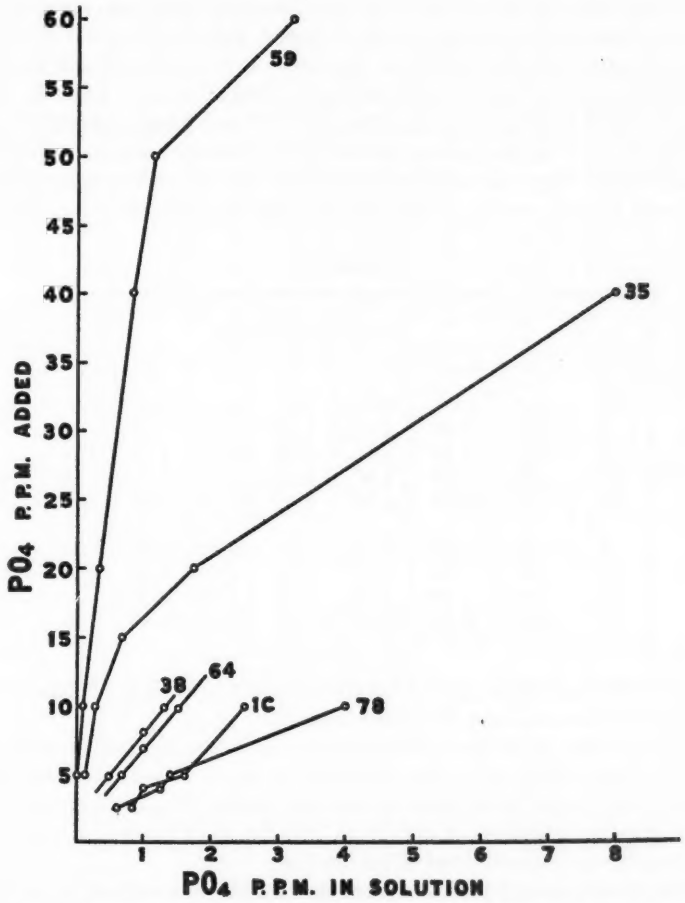

FIG. 1. FIXATION OF PO_4 FROM DIFFERENT AMOUNTS ADDED TO 100 GM. SOIL

extract and may be a very tedious procedure. The first 100 or 200 cc. of percolate may contain practically no PO_4. In table 3 are recorded the results obtained on a few soils by this method. It is so difficult to control and to produce duplicate results that this percolation method is considered very unsatisfactory for measuring fixing power. When the phosphate was applied as a dry

salt on top of the column of soil in the percolator, the results are still less useful. The leaching water or the dilute solution applied soon removes nearly all the soluble matter from the soil so that percolation nearly or entirely ceases. If some electrolyte is added with the water, percolation may be kept up, but the solubility of the PO_4 is so changed that results are of little value.

A serious difficulty with all methods for determining fixing power of a soil is that *soils have no definite fixing power*. Figure 1 and table 2 reveal that the larger the amount of PO_4 added, the less firmly is it held by the soil against the solvent action of water. Gile (9) reports similar results. Later in this paper, it will be shown that much of the added PO_4 which has apparently been rendered insoluble by the soil may be washed out by much water or by a dilute solution of Ca. Since the solubility of the PO_4 in the soil-PO_4 complex is very low, much water is needed to wash out the fixed PO_4 from the soil. Except

TABLE 3

PO_4 in successive percolates from different amounts added to 100 gm. soil

SOIL NO.	PO_4 ADDED	PO_4 IN PERCOLATE NUMBERS							TOTAL
		1	2	3	4	5	6	7	
	mgm.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1c	0	0.06	1.50	2.00	1.44	1.32	0.64	0.48	0.74
	5	0.12	1.66	2.28	2.00	2.00	1.76	1.76	1.16
	10	0.24	6.60	5.40	4.40	2.84	1.76	1.60	2.28
	20	8.00	30.80	13.50	9.00	6.16	3.20	2.68	7.33
64	0	0.24	0.72	0.88	0.88	0.84	0.64	0.56	0.48
	10	0.08	0.88	0.88	0.64	0.96	0.56	0.56	0.47
	25	3.64	7.20	5.40	5.00	3.64	2.28	2.68	2.98
	50	10.00	33.50	15.50	12.00	6.16	4.00	3.64	8.28

when the dilution is great, the soil has greater power to retain the PO_4 than the water has to dissolve it away from the complex.

Fixation of PO_4 by a soil is a time reaction which may continue for months, the rate being influenced by the proportion of water to soil. Scarseth and Tidmore (18, p. 141) found that the reaction between PO_4 and colloidal clay was practically instantaneous and (19, p. 154) that the PO_4 added to two clay soils in pots was nearly all fixed within one year.

Table 4 and figure 2 present figures showing the rate of fixation in soils kept at optimum moisture for one year, and table 5 shows the increase in fixation in a 1:1 water suspension during 20 days. The gradual decrease of water-soluble PO_4 may be partly because no definite compound, but only a loose complex, is formed, and partly because the added PO_4 makes imperfect contact with the soil particles. Perhaps 90 per cent of the PO_4 added to a soil-water mixture may be fixed in one hour (see table 5). Thus the rate of fixation is decreased in two ways, by diminishing the avidity of the soil for PO_4 and also

TABLE 4

Time effects in fixation at optimum moisture

Treatments: A = $CaH_4(PO_4)_2$, B = $CaHPO_4$, C = $(NH_4)_2HPO_4$

SOIL NO.	TIME	PO_4 IN EXTRACTS								
		Truog Extracts			1/100 Water			1/1 Water		
		A	B	C	A	B	C	A	B	C
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1c	1 hour	2.00	2.00	2.00	0.72	0.62	0.58	2.66	0.28	1.14
	1 day	2.20	2.20	1.82	0.58	0.54	0.44	1.26	0.62	0.66
	7 days	2.00	2.00	2.00	0.34	0.32	0.28	0.72	0.50	0.48
	21 days	2.00	2.00	2.00	0.36	0.26	0.28	0.80	0.72	0.62
	2 months	1.60	1.60	1.60	0.20	0.18	0.20	0.88	0.62	0.62
	6 months	2.00	1.82	1.66	0.24	0.22	0.20	0.88	0.44	0.40
	12 months	1.14	2.00	2.00	0.16	0.20	0.20	0.58	0.48	0.50
35	1 hour	0.88	0.80	0.58	0.88	0.80	0.50	1.60	1.14	0.58
	1 day	0.80	0.62	0.44	0.40	0.32	0.20	0.44	0.50	0.20
	7 days	0.58	0.48	0.40	0.14	0.12	0.08	0.16	0.16	0.12
	21 days	0.50	0.40	0.30	0.08	0.08	0.08	0.20	0.12	0.04
	2 months	0.32	0.24	0.18	0.10	0.08	0.08	0.08	0.08	0.04
	6 months	0.40	0.26	0.20	0.04	0.08	0.06	0.12	0.08	0.06
	12 months	0.28	0.30	0.20	0.08	0.10	0.04	0.04	0.08	0.08
37	1 hour	2.00	1.60	1.60	1.34	0.20	1.00	6.66	0.54	8.00
	1 day	1.60	1.42	1.66	0.88	0.24	0.62	6.66	0.72	6.66
	7 days	1.82	1.60	1.42	0.66	0.24	0.58	6.66	1.60	5.00
	21 days	2.00	1.60	1.60	0.66	0.24	0.50	6.66	2.22	4.44
	2 months	1.76	1.34	1.60	0.50	0.24	0.32	5.80	2.50	3.34
	6 months	1.82	1.42	1.42	0.58	0.30	0.28	6.66	2.00	2.26
	12 months	1.48	1.60	1.48	0.40	0.28	0.36	4.00	2.66	2.50
59	1 hour	2.00	1.60	1.00	2.22	1.60	1.00	2.86	2.36	0.88
	1 day	1.76	1.34	0.66	1.00	1.00	0.48	1.34	1.42	0.50
	7 days	1.14	0.72	0.54	0.48	0.40	0.16	0.62	0.54	0.18
	21 days	1.00	0.62	0.34	0.40	0.24	0.12	0.28	0.24	0.08
	2 months	0.58	0.50	0.20	0.12	0.14	0.04	0.12	0.10	0.06
	6 months	0.54	0.26	0.14	0.08	0.04	0.04	0.08	0.10	0.04
	12 months	0.30	0.28	0.16	0.08	0.08	0.04	0.12	0.04	0.08
64	1 hour	1.34	1.50	1.50	1.00	0.72	0.72	4.00	1.76	1.82
	1 day	1.50	1.14	1.26	0.66	0.50	0.50	2.00	1.34	1.14
	7 days	1.42	1.14	1.34	0.48	0.32	0.32	1.60	0.62	0.54
	21 days	1.34	1.00	1.14	0.36	0.20	0.24	1.14	0.48	0.34
	2 months	0.88	0.88	1.00	0.20	0.18	0.16	0.54	0.26	0.20
	6 months	1.34	1.00	1.00	0.20	0.14	0.14	0.44	0.20	0.16
	12 months	1.00	1.00	1.06	0.20	0.20	0.16	0.28	0.22	0.20
78	1 hour	0.50	0.28	0.44	0.50	0.32	0.32	2.22	1.00	0.80
	1 day	0.32	0.20	0.20	0.26	0.12	0.14	0.80	0.50	0.36
	7 days	0.30	0.20	0.20	0.16	0.08	0.12	0.44	0.14	0.18
	21 days	0.20	0.12	0.16	0.08	0.08	0.08	0.40	0.16	0.14
	2 months	0.20	0.16	0.14	0.08	0.08	0.08	0.28	0.16	0.10
	6 months	0.22	0.16	0.16	0.08	0.08	0.08	0.24	0.10	0.10
	12 months	0.10	0 20	0.10	0.08	0.08	0.08	0.18	0.12	0.12

by lowering the concentration of the uncombined PO_4 remaining in the solution. Perhaps, if the concentration in the solution were kept constant, which is very difficult to do, the rate of fixation would be constant for some time. After 90 per cent of the PO_4 is fixed, five or ten hours may be needed to fix 90 per cent of the remainder, thus leaving only 1 per cent of the original still in solution, so

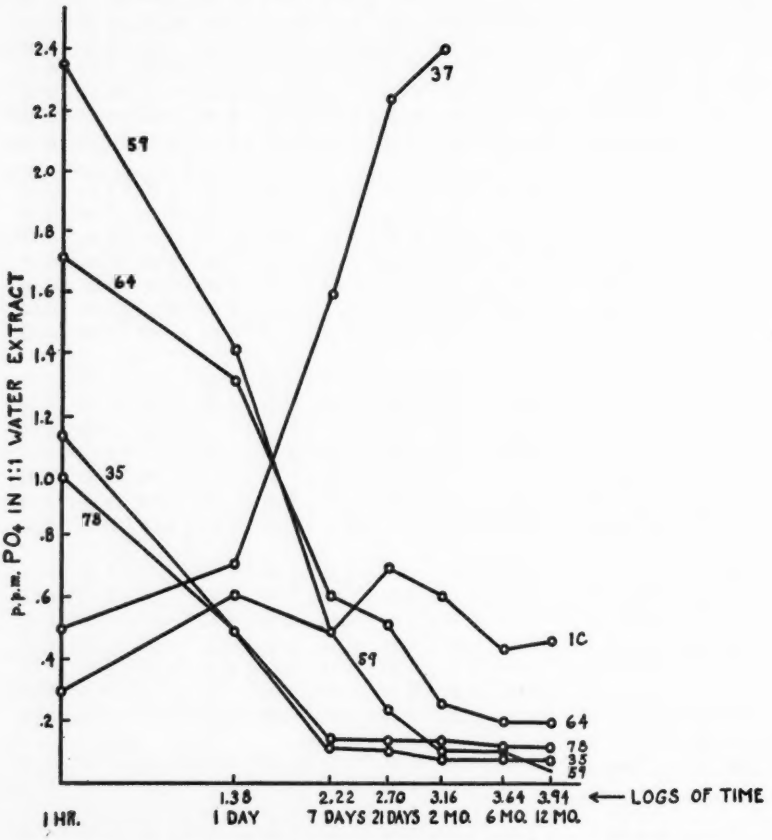

FIG. 2. CHANGE, DURING ONE YEAR, IN PO_4 IN 1:1 WATER EXTRACT OF SOILS TO WHICH WAS ADDED $CaHPO_4$

that after that the rate of fixation becomes very slow. A consequence of this time factor in fixation is that in order to obtain comparable results in any short laboratory method for measuring fixation, it is necessary to hold to a constant time during which fixation is allowed to take place. Apparently two soils having the same total fixing power may have quite different rates of fixation, on account of physical and chemical differences. The results given in table 5

show that in a 1:1 soil-water mixture, most of the fixation takes place within one hour. Since the procedure is empirical, one hour has been selected as long enough for nearly complete fixation and short enough for a convenient laboratory test.

To learn something of the rate of fixation and the length of time during which fixation takes place, the following experiment was made.

Fixation of PO_4 during one year in soil at optimum moisture.—The air-dry soils were mixed with the dry powdered phosphates, then moistened to optimum by atomizing the water onto them, and stored in covered glass jars. After

TABLE 5

Time factor in fixation from 1 minute to 20 days, PO_4 in 1:1 equilibrium extracts

TIME	FILTRATE NO.	PO_4 IN FILTRATES			
		Soils.....1c *PO_4 added, mgm.* 4	38 8	64 8	78 4
		mgm.	*mgm.*	*mgm.*	*mgm.*
1 hour	1	0.33	0.44	0.29	0.31
	2	0.88	0.80	0.88	0.50
	3	1.25	1.18+	1.25	0.63
5 hours	1	0.22	0.24	0.12	0.11
	2	0.50	0.57	0.40	0.20
	3	1.00	1.00	0.62	0.33
24 hours	1	0.22	0.36	0.13	0.06
	2	0.57	0.63	0.33	0.12
	3	1.11	1.00	0.54	0.25
5 days	1	0.25	0.16	0.06	0.04
	2	0.29	0.25	0.08	0.07
	3	0.44	0.36	0.14	0.10
20 days	1	0.12	0.16	0.06	0.04
	2	0.13	0.18	0.06	0.04
	3	0.29	0.25	0.08	0.04

some months, more water was added in order to bring the soils to the original water content. When the soils were to be sampled for analysis, the contents of the jars were well mixed before the desired portion was removed. This procedure somewhat resembles the conditions in field soils, but is not so favorable to rapid fixation as were most of the other experiments described in this paper where the soils were mixed with much more water.

Three different phosphates were used in quantity to supply the same amount of PO_4 to each jar of the same soil, but different for the different soils, with the intention that the amount added would produce a concentration of 1 p.p.m. in the 1:1 water extract at the start. The results recorded in table 4 show

that there was much departure from this. The same amount of PO_4 from different salts did not produce the same level of PO_4 in the extract.

In the following the progress of fixation, in order to obtain a better idea of the availability of the PO_4 three different extracts were made from each soil each time they were analyzed. The figures given in table 4 reveal some irregularities, perhaps partly due to inaccuracies in estimation of the PO_4 in the solutions. However, the general decrease in solubility with time is apparent, with the exception that there was an increase in the PO_4 in the 1:1 water extract of soil 37 + $CaHPO_4$. No explanation of this variation is apparent. It is evident that the amount of PO_4 fixed in a unit of time was much greater at first and gradually decreased during the year of the experiment. In figure 2 are plotted the results from the 1:1 water extract of soils + $CaHPO_4$ (given in table 4), in which the logarithms of the time units are used in order to compress the graphs into the limits of a single page. Probably a large part of the decrease in rate of fixation was caused by the great decrease of water-soluble PO_4 remaining in the soil as fixation progressed.

The amount of PO_4 fixed from the different salts was different, as indicated by the water extracts, though the acid Truog extract shows less difference. The acid $CaH_4(PO_4)_2$ was least fixed, and the neutral $CaHPO_4$ most fixed in the majority of cases, yet in some cases the very soluble ammonium phosphate was most fixed. It appears, therefore, that the relative fixing power of different soils is different for different salts, thus indicating that the most effective salt for one soil may not be the best for a different soil. The colorimetric method used in estimating PO_4 in the extracts is not capable of exact estimates of the concentration when it is below 0.1 p.p.m. in the solution, so that some variation in the smaller amounts found is not significant.

The experiment demonstrates that most of the added PO_4 was fixed within a few hours and that fixation may continue for a year or more.

Another simple experiment gives some idea of the speed of fixation. To each of four bottles containing 100 cc. water with 5 mgm. PO_4 in each were added 50 gm. soil. After being shaken for various lengths of time, the mixtures were filtered and PO_4 was determined in the extracts, with the following results:

Time before filtering	*1 min.*	*10 min.*	*1 hour*	*8 hours*
PO_4 in extract Soil 38, p.p.m.	2.00	1.44	1.16	0.88
PO_4 in extract Soil 64, p.p.m.	4.44	2.40	1.32	0.88

The greater the volume of water mixed with a soil, the greater will be the amount of PO_4 remaining in solution (table 6). In many cases, the soil extract will have almost the same concentration of PO_4 whether the ratio soil-water is 1/1 or 1/100. In other cases, when there is much easily water-soluble PO_4, the concentration of the PO_4 diminishes rapidly as the dilution increases (soils 30 and 53, table 6). In this case, since the phosphate is easily soluble, the solution is not saturated except at small dilutions. In the former case, the phosphate complex is so slightly soluble that the solution is saturated with it

TABLE 6

Increase in dissolved PO_4 with increased dilution in equilibrium water extract

SOIL NO.	DILUTION RATIO SOIL: WATER	PO_4 ADDED TO SOIL	PO_4 IN EXTRACT
		p.p.m.	*p.p.m.*
64	1/2	100	1.82
	1/5		2.10
38	1/2	100	1.25
	1/5		1.60
30	1/1	0	5.70
	1/5		3.60
35	1/2	200	1.00
	1/5		1.14
53	1/1	0	4.44
	1/5		1.34
59	1/2	400	0.62
	1/5		0.58
78	1/1	40	0.88
	1/5		0.88

TABLE 7

Fixation of PO_4 by various sizes of soil particles

SOIL	PARTICLE SIZE	PER CENT OF WHOLE	TRUOG AVAILABLE	FOR 20 GM. MATERIAL	
				PO_4 Added	PO_4 Fixed
				mg.	*mg.*
35	On 1-mm. sieve	12.2	20	2.5	1.62
	On 40 mesh	17.4	28	2.5	2.10
	On 100 mesh	18.2	36	2.5	2.42
	Through 100 mesh	48.5	56	2.5	2.48
77	On 1-mm. sieve	12.0	64	1.4	1.13
	On 40 mesh	17.3	80	1.4	1.17
	On 100 mesh	31.7	112	1.4	1.29
	Through 100 mesh	39.0	232	1.4	1.34
78	On 1-mm. sieve	0.4	...	...	.....
	On 40 mesh	0.7	...	...	.....
	On 100 mesh	13.2	48	0.8	0
	Through 100 mesh	86.7	48	0.8	0.24
95	On 1-mm. sieve	11.2	32	0.2	0
	On 40 mesh	11.3	48	0.2	0.024
	On 100 mesh	19.5	80	0.2	0.024
	Through 100 mesh	47.8	248	0.2	0.068

except at very high dilution. A consequence of these facts is that in making a test of fixing power, the ratio of soil to water must be held constant in order to obtain comparable results with different soils.

The physical character of the soil greatly influences fixation but, since this must be accepted as it is found, it need not be considered in measurement of fixation, except to see that the sample fairly represents the soil in question. If the soil is sandy and coarse, it may easily segregate into separates of different degrees of fineness which will have quite different fixing power. Figures in table 7 illustrate this very well, showing the great variations in fixing power of coarse and fine particles of four soils.

Phenomena of fixation

Time of contact, ratio soil:water, and physical character have much influence; therefore, in measuring fixation these factors must be considered and kept as nearly constant as possible. Since they have been discussed at some length under the preceding section, they may be omitted here with the reminder that they are important.

In most of the work here reported with equilibrium extracts, they were made by mixing the soil, water, and other materials in bottles, which were shaken vigorously every few minutes for an hour, then filtered on büchner filters with suction. The first portion of the filtrate, usually not clear, was returned to the filter so that a clear filtrate was obtained. The PO_4 concentration was measured by the molybdenum blue method, according to Parker and Fudge (17). In most cases where successive percolates or filtrates were made, they were obtained by adding 100 cc. of the solvent at a time to the soil on the büchner after the water of the first mixture had been sucked out as much as possible. In each case, the filter was transferred to a clean bottle to collect the new filtrate.

In most cases, the added phosphate was a water solution of KH_2PO_4 of which 1 cc. = 1 mgm. or (sometimes 10 mgm.) PO_4. The results reported and the conclusions reached in this paper are mostly based on work with equilibrium extracts. Numerous attempts to obtain useful results by means of percolation extracts produced contradictory figures, as already referred to. Perhaps, in order to obtain reliable information, it may be necessary to carry the work into the field and operate with soils in place.

Solubility in water and in salt solutions.—Though there is great difference in the solubility of the PO_4 in different soils, it is generally true that up to three or four successive filtrates of 100 cc. each from a single portion of soil, the concentration of PO_4 increases, while, at the same time, the concentration of Ca diminishes. This is shown in many of the tables, and seems to indicate that removal of easily soluble Ca releases more PO_4 to enter solution, or that concentration of PO_4 in the solution of those soils is largely governed by the concentration of Ca. The pH of the successive extracts remains nearly constant (not shown in the tables). A decrease in pH is generally accompanied by an increase of PO_4 in the solution.

Removal of soluble matter by washing the soil usually causes deflocculation to such an extent that filtration becomes very slow. In order to escape this difficulty, a solution containing about 10 p.p.m. Ca + 5 p.p.m. Mg as sulfates has been much used in this work. With this wash, it is possible to continue percolation almost indefinitely. This concentration of Ca and Mg, however, is enough to repress the solubility of PO_4 considerably.

The effect on solubility of PO_4 of the common cations of soil solutions was studied with these elements present in equivalent amounts, about 2 m.e. in

TABLE 8

Effect of 2 m.e. of several cations, etc., on PO_4 in 1:1 equilibrium extracts of soils

K_2HPO_4, in amounts indicated, was added to all except those in column 4, water only

1	2	3	PO_4 IN EXTRACTS									
			4	5	6	7	8	9	10	11	12	13
SOIL	PO_4 ADDED	FILTRATE, NO.	Water only No PO_4	Water + PO_4	Ca 11 mgm.	Mg. 6.8 mgm.	K 21.8 mgm.	Na 12.8 mgm.	NH_4 10.1 mgm.	$CaCO_3$ 0.5 gm.	0.5 gm. $CaCO_3$ + 10.1 mgm. NH_4	$MgCO_3$ 0.5 gm.
	mgm.		*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
78	4	1	0.06	0.64	0.20	0.44	0.48	0.64	0.80	0.06	0.20	0.12
		2	0.10	1.16	0.29	0.52	0.64	0.88	1.00	0.08	0.24	0.14
		3	0.14	2.00	0.40	0.72	1.44	3.20	2.16	0.06	0.24	0.16
		4	0.14	2.00	0.57	0.88	1.76	2.84	2.28	0.06	0.24	0.16
1c	4	1	0.20	1.16	0.50	1.00	0.80	1.16	1.00	0.20	0.62	0.50
		2	0.29	2.68	0.57	1.60	0.64	3.52	1.24	0.29	1.14	0.58
		3	0.57	4.00	0.80	3.20	3.20	5.32	3.68	0.29	1.60	0.66
		4	0.67	5.00	1.33	4.72	4.72	5.32	4.72	0.29	1.82	0.28
38	8	1	0.12	0.64	0.80	0.64	0.88	1.00	1.16	0.22	0.80	0.34
		2	0.50	1.32	1.16	1.76	1.60	2.00	1.32	0.29	0.88	0.38
		3	0.88	1.24	2.00	2.84	2.00	4.00	2.28	0.29	1.00	0.40
		4	1.18	1.16	2.28	2.28	2.28	2.68?	2.96	0.29	1.00	0.48
64	8	1	0.06	0.56	0.40	0.48	0.48	0.24	0.48	0.08	0.62	0.18
		2	0.12	2.16	0.80	0.88	0.72	1.60	1.44	0.08	0.66	0.16
		3	0.20	2.84	1.60	1.32	1.60	2.68	2.28	0.08	0.72	0.18
		4	0.25	3.82	1.44	1.60	2.28	2.68	2.28	0.08	0.72	0.18

100 cc., as sulfates. Results are given in table 8. It appears that solubility of PO_4 increases in the order Ca, Mg, K, Na, NH_4. With Ca ion, solubility of PO_4 is one-quarter of that in pure water, whereas with Na and NH_4, it is about the same as with water only.

The effect of $CaCO_3$ and $MgCO_3$ is also shown in table 8. $CaCO_3$ is more depressing than $MgCO_3$, but both greatly reduce solubility of PO_4. When NH_4 is added, PO_4 becomes more soluble even in the presence of $CaCO_3$ or $MgCO_3$. The NH_4 ion generally increases the solubility of PO_4 as shown in table 8 and

other tables. When Na and K are added with NH_4, the solubility of PO_4 is still more increased.

Soluble silica, added as Na_2SiO_3, seems always to increase solubility of PO_4, as shown in table 9. It appears to have a replacing or substituting power by which PO_4 is set free from the complex, as suggested by Demolon (2) and others.[3]

Other salts added with Na_2SiO_3 seem to act as they do with ammonium, as already indicated, increasing the solubility of PO_4 or not, independently of the presence of soluble silica.

Lowering of pH as by addition of an acid, or by other means, generally increases the solubility of PO_4 as the pH becomes lower, but there is much difference between different soils in this respect (see table 10). In some cases where the soil has high buffer power and very little acid is added so that pH is not much changed, the solubility of PO_4 may be decreased, through bringing into solution more Ca or Mn by the added H^+, as shown in table 10, soils 35 and 64. Teakle (20, p. 152) and Fudge (6) report the same thing.

TABLE 9

Fixing power of soil 35 as affected by several reagents

100 gm. soil in 100 cc. solution + 15 mgm. PO_4

FILTRATE NO.	PO_4 FIXED WITH 0.1 *N* HCl				PO_4 FIXED WITH Na AS Na_2SO_4		
	0 cc.	5 cc.	10 cc.	20 cc.	10 mgm.	20 mgm.	40 mgm.
	p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
1	0.36	0.20	0.16	0.12	0.24	0.24	0.24
2	0.40	0.32	0.40	0.28	0.32	0.32	0.36
3	0.66	0.50	0.58	0.40	0.50	0.58	0.62

FILTRATE NO.	PO_4 FIXED WITH NH_4 AS $(NH_4)_2SO_4$				PO_4 FIXED WITH $Na_2SiO_3 \cdot 5\ H_2O$		
	10 mgm.	20 mgm.	40 mgm.		50 mgm.*	100 mgm.*	100 mgm.†
	p.p.m.	*o.p.m.*	*p.p.m.*		*p.p.m.*	*p.p.m.*	*p.p.m.*
1	0.24	0.04	0.28		0.24	0.40	0.20
2	0.28	0.08	0.40		0.36	0.80	0.58
3	0.40		0.72		0.80	1.34	0.88

* 1 hour before filtering. † 4 hours before filtering.

Effect of replacement of Ca^{++} and Mg^{++} by H^+, K^+, or NH_4^+.—It was thought

[3] Since the PO_4 in these solutions was determined by the molybdenum blue method which produces a similar blue color with silica, it may be thought that the increase in PO_4 found was really due to silica. This appears improbable for the following reason. Never more than 25 mgm. $Na_2SiO_3 \cdot 5\ H_2O$ was added to any one solution in which PO_4 was estimated by color To evaluate the effect of the SiO_3, 100 mgm. was treated as for determination of PO_4. The color was equal to that produced by 0.004 mgm. PO_4, so that the effect of 25 mgm. $Na_2SiO_3 \cdot 5\ H_2O$ was negligible.

that removal of easily soluble Ca and other ions causing fixation, by washing the soil with water, would decrease its fixing power. The first percolate from the previously washed soil contains much more PO_4 than that from the unwashed soil, indicating much less fixation by the washed soil. But after the first percolate, the situation is reversed; the unwashed soil gave up more PO_4

TABLE 10

Effect of increasing concentration of hydrogen ion on pH and PO_4, dissolved

(50 gm. soil + PO_4 + H_2SO_4 in 100 cc. equilibrium extracts)

SOIL	PO_4 ADDED	0.1 *N* H_2SO_4	pH	PO_4 IN EXTRACT
	mgm.	*cc.*		*p.p.m.*
1c	0	0	7.2	0.20
		5	6.2	0.40
		10	5.5	0.80
		20	4.5	1.67
38	0	0	6.7	0.20
		5	6.2	0.20
		10	5.5	0.29
		20	4.5	0.57
	2	0	7.0	0.44
		5	6.0	0.44
		10	5.5	0.50
		20	4.6	1.00
64	0	0	7.0	0.04
		5	6.0	0.04
		10	5.5	0.08
		20	4.5	0.36
	2	0	7.0	0.20
		5	6.0	0.20
		10	5.4	0.29
		20	4.5	0.67
35	0	0	5.5	0.03
		5	5.0	0.03
		10	4.5	0.06
		20	4.3	0.06
	8	0	5.5	0.40
		5	5.0	0.29
		10	4.5	0.20
		20	4.3	0.20

to the wash water than did the washed soil, so that the total PO_4 in four percolates from the unwashed soil is greater than from the washed soil in the case of soil 35, and about the same with soil 64.

Replacement of the high fixing cations, Ca and Mg, by H or K was expected to reduce the fixing power of the soil, and this seemed to be true, but on account

of deflocculation of the soil, the results are in question. In one experiment all the other bases of four soils were replaced by leaching with ammonium acetate, which was then washed out with methanol, and the fixing power of the treated soil was determined. So much humus dissolved that it was necessary to remove it by ignition before estimating PO_4 in the extracts. The PO_4 found was much more than would be obtained from the untreated soils to which the same amount of PO_4 had been added. But whether the increased PO_4 was from the extracted humus or was due to decreased fixation could not be determined. Replacement of all other cations by H_2SO_4 was tried, and here the deflocculation of the treated soil was so great that the effect on solubility of PO_4 could not be determined. All attempts to determine the fixing power of soils that had been deprived of their usual cations failed. However, the effect of the removal of soluble Ca from the soil in increasing the solubility of the PO_4 is shown by the following experiment. Samples of surface soil were taken from the same spot in a garden before and after rain. The analysis gave these results:

Date of Sampling	*p.p.m. in 1:1 water extracts*		
	PO_4	Ca	SO_4
October 15, after long dry time	2.2	50	80
November 3, after 3 inches rain	2.4	40	50
January 6, after 2 inches rain	2.6	35	40
March 3, after 2 inches rain	2.7	35	30

It is evident that downward leaching of the Ca, etc., had reduced the concentration of cations so that more PO_4 could dissolve in the soil solution.

Repeated wetting, followed by drying, is sometimes supposed to render soil PO_4 more insoluble. Results of an experiment to test this theory are given in table 11. The soils were mixed with $CaH_4(PO_4)_2$ and water enough to make them very muddy, then dried in a thin layer in open pans in the air. When dry, they were pulverized, mixed, and again wetted and dried. Finally the PO_4 in the dry soils was determined in the Truog extract and in 1/100 and 1/1 water extracts.

With soils 1c and 95, of relatively low fixing power, there was little change after the first drying. Soils 35 and 77 have greater fixing power, and in them repeated wetting and drying seem to have decreased the water-soluble PO_4.

The fixing power of a number of materials other than soil is recorded in table 12. Many substances, organic and inorganic, are able to fix PO_4; how, is not clear. Permutite, presumably a sodium zeolite without Ca, has very high fixing power; perhaps an exchange of SiO_3 for PO_4 takes place. Washed beet pulp, nearly pure organic, seems to have a negative fixing power. When dry, it takes up water faster than the PO_4 dissolved in the water so that the concentration of PO_4 in the remaining water is increased.

Relation of PO_4 to Fe and Ca dissolved by dilute H_2SO_4.—The PO_4, Fe, and Ca extracted from four soils by digestion with dilute H_2SO_4 for three days is shown in table 13. When the solvent was 0.005 *N* acid, the PO_4 dissolved was more

TABLE 11

Effect of repeated wetting and drying on fixation of PO_4

SOIL	TIMES DRIED	PO_4 IN SOLUTION		
		Truog extract	Water extract	
			1/100	1/1
		p.p.m.	*p.p.m.*	*p.p.m.*
1c	1	3.3	0.88	1.44
	2	3.3	0.84	1.24
	3	3.3	0.80	1.24
35	1	1.44	1.00	1.00
	2	1.32	0.72	0.68
	3	1.60	0.72	0.56
77	1	1.76	1.08	1.76
	2	1.76	0.96	1.76
	3	1.76	0.96	1.32
95	1	1.32	0.80	8.80
	2	1.32	0.80	8.80
	3	1.32	0.80	8.80

TABLE 12

Fixation of PO_4 by various substances

SUBSTANCE	AMOUNT	PO_4	
		Added	Fixed
	gm.	*mgm.*	*mgm.*
Delta peat	20	2.0	0.886
German peat	10	1.0	0.500
Garden compost	20	1.0	0
Wood flour, spruce	10	1.0	0.42
Permutite	20	15.0	14.908
Kaolin	20	1.0	0.80
Diatomaceous earth	20	1.0	0
$BaSO_4$, very fine	20	1.0	0
Talc, powdered	20	1.0	0.4
Filter paper	10	1.0	0.2
Beet pulp (washed)	10	5.0	0
Talc	20*	0.12	0.06
Pumice	20*	0.12	0.048
Feldspar	20*	0.12	0
Mica	20*	4.00	3.76
Infusorial earth	20*	0.40	0.336

* Through 100-mesh sieve.

than equivalent to the Fe dissolved from soils 35, 59, and 64. But when the acid was 0.01 *N*, the iron dissolved was in all cases more than equivalent to the PO_4, and with stronger acid a still larger proportion of Fe was dissolved. Excess Ca was always dissolved. It is inferred that the PO_4 dissolved by the .005 *N* acid was probably present in the soil in combination with Ca. But the excess Fe dissolved by the stronger acids may indicate that most of the PO_4 in the soils was held in combination with iron. Heck (10) offers some information on this point. Dean (1) finds electrodialysis a useful means of obtaining information in regard to the phosphate compounds in soil. He

TABLE 13

PO_4, Fe and Ca in acid extracts of soils

2 gm. soil in 200 cc. solution, shaken 2 days

SOIL	NORMALITY OF H_2SO_4	AMOUNT DISSOLVED		
		PO_4	Fe	Ca
	-	*m.e.*	*m.e.*	*m.e.*
35 (pH 5.4)	0.005	0.012	0	....
	0.010	0.028	0.14	....
	0.050	0.120	5.3	.....
	0.100	0.200	13.2	....
59 (pH 6.0)	0.005	0.001	0	0.3
	0.010	0.004	0.017	0.7
	0.050	0.025	0.190	1.0
	0.100	0.062	0.648	1.2
64 (pH 7.0)	0.005	0.028	0.014	3.0
	0.010	0.026	0.130	7.0
	0.050	0.106	2.140	25.0
	0.100	0.214	2.970	25.0
78 (pH 4.8)	0.005	0.008	0.024	0
	0.010	0.016	0.071	0
	0.050	0.034	0.324	0
	0.100	0.064	0.400	0

agrees with Heck. Iron phosphate complexes in soil are not well decomposed by this method, nor are the very insoluble basic phosphates of some calcareous soils. But the ordinary calcium phosphates of non-calcareous soils readily give up their PO_4 by electrodialysis.

Causes of fixation

Results given in the preceding section, "Phenomena of Fixation," indicate some of the causes. The effect of iron and aluminum colloids has been well dealt with by Gile (9) and others mentioned in the introduction, and was little considered in the present study.

The influence of pH is most important, since phosphates of Ca and Mg become easily soluble below pH 7 whereas Fe and Al phosphates are not much dissolved above pH 3. But colloids of Fe and Al may fix PO_4 at pH 3–8. Above pH 8, they may hydrolyze, so that PO_4 becomes soluble in alkaline solutions though Fe does not. Below pH 7 added Ca^{++} or Mg^{++} depresses solubility of PO_4 and above that makes it nearly insoluble. Removal of Ca from the solution greatly increases solubility of PO_4 as shown by Teakle (20, p. 147). $CaCO_3$ and $MgCO_3$ raise pH and increase Ca^{++} and Mg^{++} so that PO_4 is only slightly soluble in their presence. The finer the particles of soil the greater is their fixing power, as shown in table 7.

The composition of the complex particles of the soil must greatly influence fixation. If they are inert like quartz, they will have little fixing power, but reactive substances like kaolin or mica may have considerable effect. Fixation by a number of substances, both organic and mineral, is shown in table 12. It appears that diatomaceous earth—which is mostly silica—, feldspar, and barium sulfate, have no fixing power. Talc, mica, kaolin, and an impure infusorial earth have considerable fixing power. Most fixation is probably the result of chemical action, yet some must be due to physical forces located in or on the surfaces of the particles of such substances as talc, kaolin, peat, and filter paper (*see* table 12). Whether 90 per cent of fixation is due to chemical action, as indicated by Teakle (20, p. 160), is questionable. Doughty (3) has shown that the organic matter of peat has much fixing power. The fixing power of colloidal iron and aluminum compounds has been found great by Gile (9), and both he and Scarseth and Tidmore (18, 141) found that the fixing power of soil colloids varies inversely as the ratio $\frac{SiO_2}{R_2O_3}$ of the colloid. Soil 59 used in the present study has the highest fixing power of any studied and it has the greatest proportion of hydrated iron oxide.

Control of fixation

In a practical way, it is very desirable to know how to treat a soil to increase or decrease fixation.

Increase of fixation is caused by raising the pH; by adding $CaCO_3$ or other carbonates or solutions of Ca, Mg, and Fe; and by increasing colloidal matter, such as by deflocculating.

Decrease of fixation is brought about by reversing these processes and also by adding ammonium or sodium salts or silica solutions.

Probably one of the best methods for getting soluble phosphorous deeply into the soil is to add it in some easily decomposed organic compound or in a colloidal complex not subject to fixation and wash it down by flooding.

Changes in fixation caused by addition of several substances is summarized in table 14.

Placement of phosphate in the soil in the most favorable location for absorption by the roots may considerably modify the effect of fixation. Hockensmith

(11) and his associates found that favorable placement of the fertilizer largely increased absorption by plants. Gericke (8) made a considerable study of this factor and found that concentration of the added superphosphate in a zone of 5 cm. thickness at 20–25 cm. depth gave best results with cereals, wheat, oats, rye, barley. This increased the yield 13–27 per cent over that obtained when the fertilizer was uniformly distributed through the upper 30 cm. of soil. The chief difficulty is to place the phosphate in the most favorable location, which is largely dependent on the fixing power of the soil.

Midgley (16) and others have found it advantageous, in the case of high fixing soils, to apply the fertilizer in relatively large sized particles, in which

TABLE 14

Increase or decrease of fixing power caused by addition of various substances

50 gm. soil in 100 cc. solutions, shaken 1 hour, filtered on Büchner, and washed with 10 p.p.m. Ca and 5 p.p.m. Mg

SOIL	FILTRATE NO.	PO_4 ADDED	PO_4 IN FILTRATES AFTER ADDITION TO SOIL OF				
			H_2O only	$CaCO_3$ 1 gm. + PO_4	$CaSO_4$ 0.23 gm. + PO_4	Na_2SiO_3 0.2 gm. + PO_4	$(NH_4)_2SO_4$ 0.05 gm. + PO_4
		mgm.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
64	1	10	0.06	4.0	2.5	4.0	...
	2		0.04	4.4	4.0	6.6	...
77	1	15	0.06	4.0	....	5.7	...
	2		0.04	8.8	....	20.0	...
95	1	1	1.24	8.8	....	8.0	...
	2		1.16	6.6	....	10.0	...
35	1	15	0.06	0.85	1.35	2.0	2.1
	2		0.04	0.65	1.10	5.0	2.7
59	1	25	0	0.72	0.66	0.50	...
	2		0	0.66	0.62	1.60	...
	3		0	0.62	0.58	2.66	...

the PO_4 remains in a soluble condition some time, instead of in fine particles, which are rapidly fixed. Scarseth and Tidmore (18, p. 161) point out the advantage of using pellets instead of mixing throughout the soil.

Deep placement of phosphate is desirable for fruit trees, but has not yet been successfully and economically accomplished.

SUMMARY

A review of the papers of ten investigations indicates that fixation of phosphate in soil in order of decreasing importance is most influenced by pH, Ca^{++}, and ratio $\frac{SiO_2}{R_2O_3}$ in the soil colloids.

A simple method is described whereby the relative fixing power of soils may be estimated and given appropriate numerical representation.

"Fixing power" is defined as the number of milligrams PO_4 per kilogram soil that must be added so that a 1:1 water extract will contain 1 p.p.m. PO_4.

The influence of time, ratio $\frac{\text{soil}}{\text{water}}$, size and composition of soil particles, and the effect of addition to the soil of H^+, Ca^{++}, Mg^{++}, K^+, Na^+, NH^+, soluble silica, and $CaCO_3$ are studied and the results discussed.

Many other substances than soil are found to have the ability to fix soluble phosphate.

Means for modifying fixation are: to increase fixation, raise pH and add Ca^{++}, $CaCO_3$, and soil colloids; to decrease fixation, do the opposite, also add Na^+, NH_4^+, soluble silica, organic colloids, organic compounds of phosphorus, employ special methods of placement, and use pellets instead of fine particles of the phosphate fertilizer.

REFERENCES

(1) Dean, L. A. 1934 Electrodialysis as a means of studying the nature of soil phosphates. *Soil Sci.* 37: 253–266.

(2) Demolon, A. 1933 Fixation and exchange of certain anions by soil colloids. *Mezögazdasági Kutatások* 6: 474.

(3) Doughty, J. L. 1930 Fixation of phosphate by a peat soil. *Soil Sci.* 29: 23–35.

(4) Ford, M. C. 1933 The nature of phosphate fixation in soils. *Jour. Amer. Soc. Agron.* 25: 134–143.

(5) Fraps, G. S. 1910–1933 Several bulletins of Tex. Agr. Exp. Sta., such as 126, 178, 212, 267, 304.

(6) Fudge, J. F. 1928 Influence of nitrogenous fertilizers on availability of phosphate. *Jour. Amer. Soc. Agron.* 20: 280–293.

(7) Gaarder, T. 1930 Fixation of phosphoric acid in soils (in German). *Medd. Vestlandets Forst. Forsokssta.* 14 (Abs. from *Chem. Abs.* 24: 5408, 1930).

(8) Gericke, S. 1934 Effects of depth of placement of phosphate on crop produced (title translated). *Die Phosphorsäure* 4: 79–97.

(9) Gile, P. L. 1933 Effect of different colloidal materials on efficiency of superphosphate. U. S. Dept. Agr. Tech. Bul. 371.

(10) Heck, A. Floyd 1934 Phosphate fixation and penetration in soils. *Soil Sci.* 37: 343.

(11) Hockensmith, R. D., Gardner, R., and Kezer, A. 1933 The effect of placement on the availability of superphosphate in calcareous soils. *Soil Sci.* 36: 35–38.

(12) Lohse, W. H., and Ruhnke, G. N. 1933 Studies on readily soluble phosphate in soils: III. *Soil Sci.* 36: 303.

(13) Lohse, W. H., and Ruhnke, G. N. 1933 Extractions of readily soluble phosphate from soils by means of dilute $KHSO_4$. *Soil Sci.* 35: 457.

(14) McCool, M. M. 1928 Determination of fixing power of soils for phosphate. *Proc. 1st Internatl. Cong. Soil Sci.* 2: 408.

(15) McGeorge, W. T., and Breazeale, J. F. 1932 Studies on iron, aluminum and organic phosphates, and phosphate fixation in calcareous soils. Ariz. Agr. Expt. Sta. Tech. Bul. 40.

(16) Midgley, A. R. 1931 Effect of placement. *Jour. Amer. Soc. Agron.* 23: 788.

(17) Parker, F. W., and Fudge, J. F. 1927 The colorimetric determination of organic and inorganic phosphorus in soil extracts and in the soil solution. *Soil Sci.* 24: 109–117.

(18) SCARSETH, G. D., AND TIDMORE, J. W. 1934 Fixation of phosphates by soil colloids. *Jour. Amer. Soc. Agron.* 26: 138–151.

(19) SCARSETH, G. D., AND TIDMORE, J. W. 1934 Fixation of phosphates by clay soils. *Jour. Amer. Soc. Agron.* 26: 152–162.

(20) TEAKLE, L. J. H. 1928 Phosphate in the soil solution as affected by reaction and cation concentration. *Soil Sci.* 25: 143–162.

(21) THOMAS, WALTER 1933 Absorbtion, utilization, etc., of N, P, and K by apple trees. *Jour. Agr. Res.* 47: 565–581.

(22) WEISER, V. L. 1933 Fixation and penetration of phosphates in Vermont soils. Vt. Agr. Exp. Sta. Bul. 356.

THE DETERMINATION OF ABSORBED BASES BY BOILING WITH AMMONIUM CHLORIDE AND THE UTILITY OF THE PROCEDURE IN RELATED SOIL INVESTIGATIONS

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In the determination of exchangeable bases in soils by methods such as those of Gedroiz (3), Hissink (4), and Kelley and Brown (5), cold ammonium chloride extractions have been used extensively. This salt is an effective exchange agent, but its advantages are offset by its solvent action upon calcium carbonate. Recently Di Gléria (2) showed that boiling ammonium chloride effected stoichemetrical decomposition of precipitated carbonates. He proposed that this treatment be used to eliminate soil carbonates in base-exchange studies and that the ammonia found in 200 ml. of distillates from boiling ammonium chloride suspensions be used to compute the carbonates. Di Gléria concluded that absorbed bases effect no liberation of ammonia from the boiling ammonium chloride solution. His proposal for the determination of soil carbonates was therefore based on the premise that the ammonia evolved is due solely to the reaction indicated by the equation:

$$CaCO_3 + 2NH_4Cl \rightarrow CaCl_2 + (NH_4)_2CO_3$$

Because of the possible adaptation of this principle to certain absorption and base-exchange investigations at this station, it seemed advisable to study the solvent action of boiling ammonium chloride upon unusual carbonate and absorbed residues from different calcic and magnesic additions to certain soils, especially several of known lysimeter history. The results of the study and the several procedures developed therefrom are given in this paper.

THE DECOMPOSITION OF LIMESTONE AND OF DOLOMITE OF VARYING DEGREES OF FINENESS IN BOILING SOLUTIONS OF AMMONIUM CHLORIDE

The first distillations were made to insure complete disintegration of substantial occurrences of both limestone and dolomite by the boiling with ammonium chloride. In studying this point, together with the speed of decomposition, 0.5-gm. charges of limestone and of dolomite were used without soil. This represents a 10-gm. charge of a soil containing 5 per cent of $CaCO_3$. The factor of degree of fineness was introduced by the use of four separates of lime-

[1] The analytical procedure was developed by the senior author. The junior author is responsible for the presentation.

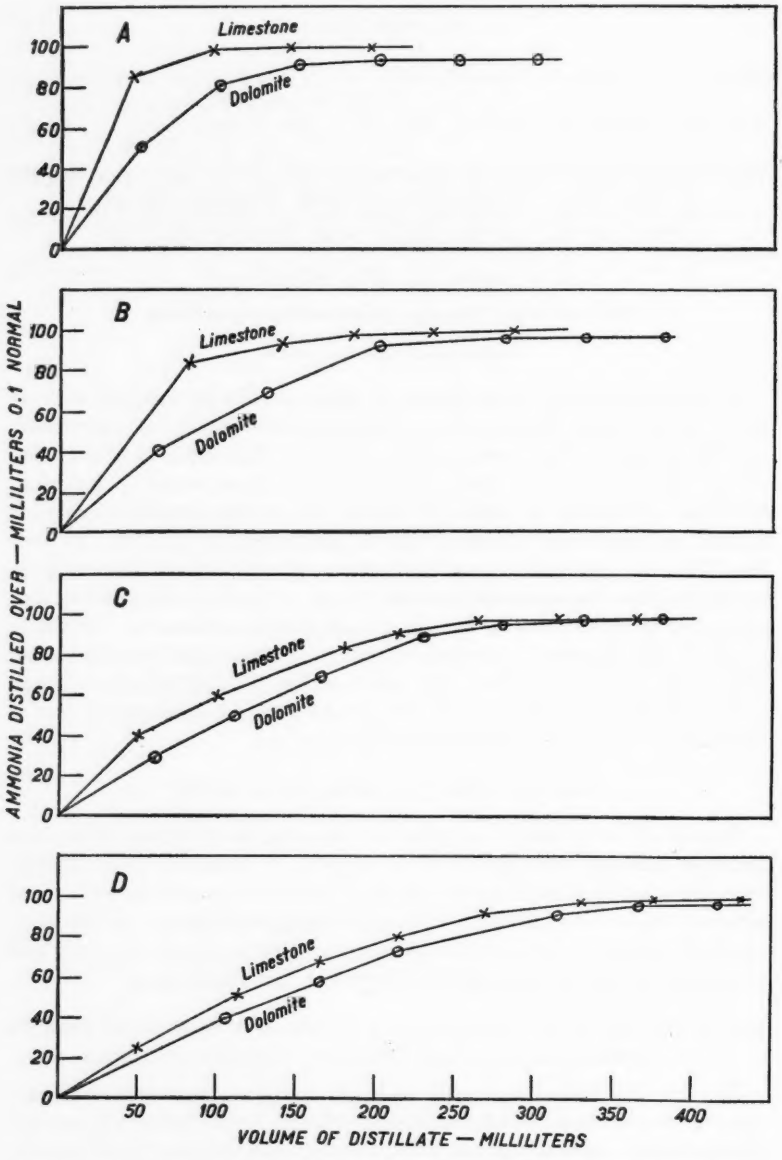

FIG. 1. DISINTEGRATION OF LIMESTONE AND OF DOLOMITE BY BOILING NORMAL SOLUTIONS OF AMMONIUM CHLORIDE, AS DETERMINED BY AMMONIA DISTILLATIONS

A—Separates through 325-mesh.
B—Separates through 200-mesh, but stopped on 325-mesh.
C—Separates through 100-mesh, but stopped on 200-mesh.
D—Separates through 60-mesh, but stopped on 100-mesh.

stone and of dolomite. To each charge were added 100 ml. of a neutral normal solution of ammonium chloride and 200 ml. of water in a distillation flask. The distillation apparatus used throughout the present work was the all-glass Pyrex unit 1370. The mixtures were boiled and the distillates delivered into a standardized acid. The ammonia contents of successive measured fractions were determined at definite intervals, and these data were used to construct the four curves of figure 1. The evolutions of ammonia were plotted against the distillate volumes. The volumes can be translated into time by assigning 200 ml. per hour as the average rate of distillation.

Curve A shows that the limestone decomposition was much more rapid than that found for dolomite. Boiling for 30 minutes was sufficient to effect complete decomposition of the 0.5-gm. charge of 325-mesh limestone, whereas 1 hour was required for complete disintegration of the corresponding charge of dolomite.

The speed of the decomposition of the 200-mesh charges is shown in curve B. The initial speed found for the limestone was again much greater than that found for the dolomite. The complete decomposition of the sample of 200-mesh limestone required about 1 hour, whereas the dolomite sample required 1 hour and 15 minutes. The decompositions shown for the 200-mesh separates were considerably less rapid than those shown for the 325-mesh separates in curve A.

The 100-mesh data are plotted in curve C. The decomposition curves found for the 100-mesh separates of the two limestones are more concordant than those shown for the finer meshes, 1½ hours being requisite for the complete decomposition of either the limestone or the dolomite.

The 60–100-mesh data are shown in curve D. The speed of disintegration of the less soluble dolomite approximated that found for limestone, the ammonia-liberation curves of the two separates of this mesh being almost parallel. Approximately 2 hours was required for the complete decomposition of the 60–100-mesh limestone and the dolomite in the boiling solution of ammonium chloride.

Hence, if 60-mesh separates are assumed to be representative of the limestone residues in field soils, or if the soil samples are reduced to that fineness, boiling for 2 hours with a normal NH_4Cl solution should effect complete decomposition of a 5 per cent calcium carbonate content of a 10-gm. charge of soil.

The relationship between the speed of decomposition of limestone and that of dolomite outside of the soil system, as influenced by the size of particles, is interesting in relation to previous observations as to the soil's capacity to effect decomposition of economic additions of limestone and dolomite separates. The carbonate residues from the coarser separates of dolomite were greatly in excess of those found for corresponding limestone additions, but this difference disappeared when separates of 80–200-mesh were used (6).

THE TENDENCY OF SOIL TO RETAIN THE NH_4 IONS DERIVED FROM BOILING AMMONIUM CHLORIDE

The next step was to determine whether the soil exerted any retentive effect upon the ammonia released from boiling ammonium chloride. In the study of this factor a very acid sandy loam, practically devoid of exchangeable bases, and a stiff clay acid subsoil that contained 5.8 m.e. of exchangeable calcium were used. Mixtures of 10-gm. charges of soil, 100 ml. of normal ammonium chloride, and 200 ml. of water were boiled, and the ammonia content of the first 200-ml. distillate was determined in 50-ml. fractions. Similar distillates were obtained from the same soils supplemented by precipitated $CaCO_3$ and also from the NH_4Cl blank.

The results given in table 1 show that neutral ammonium chloride yielded appreciable quantities of NH_4OH when boiled alone for 1 hour, the period required to obtain four 50-cc. distillates. Based on a 10-gm. charge, the

TABLE 1

Tendency of soil to retain a part of the NH_4 radical derived from ammonium chloride on boiling, with and without $CaCO_3$ additions

SYSTEM	0.1 *N* NH_4OH LIBERATED				
	1st 50 ml.	2nd 50 ml.	3rd 50 ml.	4th 50 ml.	Total 200 ml.
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
$NH_4Cl + H_2O$ only (pH 7.0)	1.80	0.60	0.30	0.15	2.85
NH_4Cl + sandy soil	0.65	0.60	0.60	0.60	2.45
NH_4Cl + subsoil	1.30	0.70	0.60	0.60	3.20
NH_4Cl + soil + 0.5 gm. $CaCO_3$	94.00	1.80	0.60	0.60	97.00
NH_4Cl + subsoil + 0.2 gm. $CaCO_3$	37.00	2.00	0.80	0.70	40.50

ammonia of the total distillate would be equivalent to 2.85 m.e. per 100 gm. of soil. When to this system was added 10 gm. of the very acid sandy loam, a small decrease in the ammonia content of the 200-ml. distillate was found. This acid soil, effecting pH decrease through exchange of its H for the NH_4 of the solution, apparently exerted a buffering effect on the rate of ammonia-release, making it nearly constant for the four fractions, whereas nearly two-thirds of the ammonia-yield from the neutral blank was in the first fraction. The acid clay subsoil also showed a buffering effect on the first fraction of the distillate.

The addition of 0.5 gm. of $CaCO_3$ to the boiled suspension of the acid soil yielded the equivalent of 97 ml. of 0.1 *N* NH_4OH, and when corrected for the soil blank, this represents a deficiency of 5.45 ml. of 0.1 normal ammonia. The addition of 0.2 gm. of $CaCO_3$ to the subsoil gave a 2.70-ml. deficiency in the ammonia yield, when corrected for the subsoil blank. The smaller deficiency from the clay soil reflects the balancing effects of the exchangeable calcium. The data of table 1 therefore demonstrate that a part of the ammonia that

was derived from the boiling solution of ammonium chloride was retained by the soil, either alone or with $CaCO_3$, and that this retentive tendency was diminished by exchangeable bases.

THE EFFECT OF ABSORBED[2] CALCIUM AND MAGNESIUM ON THE RELEASE OF AMMONIA FROM AMMONIUM CHLORIDE

To determine more specifically this influence on the release of ammonia from ammonium chloride, three soils of varying content of absorbed calcium and magnesium, with and without concomitant carbonates, were used. Each 10-gm. charge was introduced into a 400-ml. volume that included 100 ml. of *N* NH_4Cl, and a 200-ml. distillate was delivered into standardized acid. Two additional 50-ml. fractions were collected to determine the ammonia

TABLE 2

*The capacity of the absorbed calcium and magnesium of certain humid soils to liberate ammonia from a boiling solution of ammonium chloride**

SOIL NO.	PREVIOUS TREATMENT	RESIDUAL CARBONATE CONTENT	ABSORBED Ca + Mg IN EXTRACT	AMMONIA IN DISTILLATES				
				1st 200 ml.	5th 50 ml.	6th 50 ml.	Total 300 ml.	Excess over carbonate value
		m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
1. Sandy loam.....	Limestone	0.8	10.5	10.45	0.80	0.70	11.95	11.15
2. Silt loam.....	Limestone	1.6	12.5	10.40	0.70	0.70	11.80	10.20
3. Silt loam.....	Burnt lime	44.2	20.3	58.40	1.30	1.00	60.70	16.50
4. Silt loam.....	Limestone	59.6	20.4	73.50	1.50	1.00	76.00	16.40
5. Clay subsoil.....	Limestone	33.6	12.2	40.50	0.60	0.50	41.60	8.00
6. Clay loam.....	None	0	19.5	4.50	0.50	0.40	5.40	5.40
7. Clay loam.....	Magnesium carbonate†	21.8	47.0	63.90	2.20	2.10	68.20	46.40
8. Clay loam.....	Magnesium carbonate†	2.0	12.0	14.40	1.00	1.00	14.40	14.40

* Expressed as milliequivalents per 100 gm. of soil.

† Normal crystalline, $MgCO_3 \cdot 3H_2O$.

released during the final stage of distillation. The data thus obtained are given in table 2. It is evident that the ammonia liberated from the ammonium chloride is not due solely to the carbonates of calcium and magnesium. The absorbed calcium and magnesium effected a liberation of ammonia that is evidenced when the sixth column of table 2 is compared with the two preceding columns of the same table. These data indicate that the reactions involved in the liberation of ammonia from a boiling ammonium chloride solution and a soil are represented by the following equations:

[2] The expression "absorbed" will be used to connote the bases absorbed and those exchangeable (8).

$$1.\quad CaCO_3 + 2NH_4Cl \rightleftarrows CaCl_2 + (NH_4)_2CO_3$$
$$2a.\quad CaX + 2NH_4Cl \rightleftarrows (NH_4)_2X + CaCl_2$$
$$b.\quad (NH_4)_2X + 2H_2O \rightleftarrows H_2X + 2NH_4OH$$
$$3a.\quad MgX + 2NH_4Cl \rightleftarrows (NH_4)_2X + MgCl_2$$
$$b.\quad (NH_4)_2X + 2H_2O \rightleftarrows H_2X + 2NH_4OH$$

The designations CaX, MgX, $(NH_4)_2X$, and H_2X represent the soil complexes that carry the respective cations.

In a comparison of the data in the last column with those in the fifth column it is evident that the second and third forward reactions do not go to completion, and that varying quantities of the NH_4 ion are retained, depending upon the specific nature of the soil complex. The reversal of the reaction is not due to the peculiarity of the absorbed ions of calcium and magnesium as against those derived from the $CaCO_3$ and $MgCO_3$ content of the soil, but rather to the specific ability of each soil to hold the liberated ammonia at the last

TABLE 3

Retention of ammonia by soils after distillation with ammonium chloride, and subsequent removal of the solute by alcohol washings

SOIL NO.*	PREVIOUS TREATMENT	RETAINED NH_4†	DISTILLATE NH_4 IN EXCESS OF CO_2	TOTAL NH_4 RECOVERED	ABSORBED Ca + Mg
		m.e.	*m.e.*	*m.e.*	*m.e.*
1. Sandy loam	Limestone	2.1	11.15	13.25	10.5
2. Silt loam	Limestone	5.5	10.20	15.70	12.5
6. Clay loam	None	15.7	5.40	21.20	19.5

* Those of table 2.

† And yielded by subsequent distillation with MgO.

experimental stage of the distillation. When in contact with NH_4Cl, the soil will exchange its several basic ions, including H ions, for NH_4 ions; and the equations 2b and 3b indicate what transpires when a soil is present in the system with NH_4Cl solution.

This point was demonstrated by another experiment, in which several soils were distilled with NH_4Cl solution. Each residue was filtered and washed with alcohol to remove solute NH_4Cl. The NH_4 retained by each soil was then determined by adding MgO and distilling. The results thus obtained and shown in table 3 confirm the conclusion that any disparity between the ammonia content of distillates and the ammonia equivalence of total absorbed bases is not due to incomplete reaction between the NH_4Cl and the absorbed bases, but rather to the capacity of the soil complex to fix the literated ammonium ion, the extent of fixation varying with the nature of the soil complex. The total yield of ammonia, the sum of that released and carried over in the distillate and that recovered from the soil residue, is somewhat in excess of the total

of bases displaced. This is accounted for in part by the ammonia given off by the NH_4Cl blank of table 1, a factor not considered in the foregoing discussion.

The data of tables 2 and 3 demonstrate that when a soil rich in carbonates is boiled with a solution of ammonium chloride, the resultant liberation of ammonia is due, not only to carbonates, but also to *the absorbed calcium and magnesium*. This is in direct contradiction to Di Gléria's conclusion that the ammonia-liberation in the soil-NH_4Cl system is due entirely to the carbonate content of the soil, and expressed solely by the equation,

$$CaCO_3 + 2NH_4Cl \rightleftarrows CaCl_2 + (NH_4)_2CO_3 \rightarrow (2NH_3 + H_2O + CO_2)$$

The data of table 3 further indicate that the absorbed and carbonate bases were completely displaced by the NH_4 ions within the distillation periods. This is evidenced by the fact that the disparities between the ammonia contents of the distillates and the joint equivalences of the extracted carbonates and absorbed bases were fully accounted for by the subsequent recoveries of ammonia from the washed soil residues.

THE SOLVENT ACTION OF BOILING AMMONIUM CHLORIDE ON THE SOIL COMPLEX

Since complete extraction of the bases present in both the absorbed and the carbonate forms was effected by the boiling digestion and supplementary leaching, the solvent action of the boiling ammonium chloride solution was greater than that found for cold dilute acid extractions in related studies (8). But the removal of the same bases by means of a dilute acid or an acid of moderate concentration would necessitate the application of heat and this would cause considerable quantities of iron, aluminum, and silica to go into solution during the extraction process. The question then arises as to the solvent action of the boiling ammonium chloride extraction upon the amphoteric components of the soil complex. To elucidate this point, the combined filtrates and washings from the ammonium chloride digestion were neutralized with ammonia and digested to flocculate the precipitates of the iron and aluminum hydroxides, which were ignited and weighed jointly as Fe_2O_3 and Al_2O_3. Two saline Colorado soils were included in this comparison. These two soils were rich in carbonates and were heavily impregnated with salts of Ca, Mg, Na, and K, as will be seen by reference to their analyses (9). The quantities of Fe_2O_3, Al_2O_3, and SiO_2 dissolved from the soils by boiling in an ammonium chloride solution for $1\frac{1}{2}$ hours were surprisingly small for all soils, as shown by the data of table 4. The solvent action exerted by the boiling ammonium chloride on sesquioxides was only a small fraction of that previously found for cold extractions with 0.05 *N* HCl. In a related study it was determined that a 1-liter leaching with 0.05 *N* HCl failed to extract all of the calcium from the limed soils of the Pennsylvania station plats, soils 3 and 4 of table 2; nevertheless, the average amount of $Fe_2O_3 + Al_2O_3$ removed by the dilute acid solution was 0.37 per cent of the soil charge.

It should be recorded that, at the conclusion of the distillations, the liquid phase of each soil-ammonia chloride system had a pH value ranging from 4.4 to 4.6, irrespective of the wide range of pH values that obtained at the beginning of the distillation. The initial pH values of the soils ranged between 4.4 and 10.4, whereas the original solution of ammonium chloride had a constant pH of 7.0. The low solubility of alumina in the boiling solution that ultimately attained and maintained a pH value close to 4.4 is ascribed to the stabilizing effect of the NH_4 ions absorptively held by the soil complex during the digestion.

Moreover, the boiling ammonium chloride extraction exerted no determinable solvent action upon the mineral forms of calcium and magnesium that are soluble only in strong HCl. The residue of one sample that had yielded 10 m.e. of exchangeable magnesium and still contained 80 m.e. soluble in HCl of 1.115 sp. gr., was subjected to an additional 1-hour digestion with boiling ammonium chloride and the subsequent washing. The *second* boiling ammonium chloride digestion failed to extract any further determinable quantity of magnesium. But when the same soil was subjected to four 16-hour agitations with cold 0.02 *N* HCl, a continuous yield of magnesium was obtained. Hence, the single digestion with boiling ammonium chloride gave full recovery of the exchangeable magnesium, whereas the solvent effect was still manifest in the fourth extraction with the dilute acid.

TABLE 4

The solvent action of boiling normal ammonium chloride solution upon soil content of Fe_2O_3 and Al_2O_3 and SiO_2

SOILS	$Fe_2O_3 + Al_2O_3$ DISSOLVED FROM 10-GM. CHARGES	SiO_2
	gm.	*gm.*
Sandy loam, high in acid soluble Al_2O_3	0.0046	None
Silt loam, previously limed	0.0016	None
Silt loam, previously limed	0.0009	None
Acid clay, previously limed	0.0025	None
Saline dolomitic Colorado sandy loam*	0.0200	0.09
Saline calcareous Colorado clay†	0.0600	0.12

* Total solids extracted by ammonium chloride digestion 13.83 per cent; † 7.64 per cent.

THE ADEQUACY OF A 250-ML. LEACHATE-VOLUME FOR THE RECOVERY OF CALCIUM AND MAGNESIUM AFTER BOILING AMMONIUM CHLORIDE EXTRACTIONS

It seemed that the volume of ammonium chloride used in the Büchner washings could be reduced below the larger amounts used ordinarily in gravitational leachings. An untreated soil of high clay content, one of high magnesium content, and two soils of high calcium content were therefore used to determine the adequacy of 250 ml. for the digestion and washings. The soils

were all subjected to the preliminary treatment of NH_4Cl-digestion and -distillation, the residues being transferred to filters and washed with ammonium chloride. In one series the NH_4Cl filtrate and washings were limited to a combined volume of 250 ml.; in the other series an additional 250-ml. leachate was obtained. The calcium and magnesium contents of the filtrates were determined and are given in table 5.

These data show that practically the same quantities of calcium and magnesium were removed by the uniform digestion-distillation and the leachings of 250 ml. and of 500 ml. It therefore seems certain that, after the distillation treatment with normal ammonium chloride, a total volume of 250 ml. of filtrate and leachate is sufficient for the complete extraction of both the absorbed and the carbonate forms of calcium and magnesium, when the transferred digestion volume does not exceed 100 ml., i.e., when the leachate-washings are as much as 150 ml.

TABLE 5

*The adequacy of 250 ml. ammonium chloride leachates for the recovery of calcium and magnesium after boiling with ammonium chloride (e)**

SOIL	PREVIOUS TREATMENT	250-ML. LEACHATES			500-ML. LEACHATES		
		Ca	Mg	Ca + Mg	Ca	Mg	Ca + Mg
		m.e.	*m.e.*	*m.e.*	*m.e.*	*m.e.*	*m.e.*
Clay soil.....	None	11.0	10.00	21.0	11.2	10.20	21.4
Clay subsoil.....	Limestone†	45.4	0.96	46.4	45.5	1.66	47.2
Clay loam.....	Burnt lime‡	64.5	1.32	65.8	64.6	1.40	66.0
Clay loam.....	$MgCO_3$§	4.6	70.70	75.3	....	.....	74.4‖

* The final volume of the digestion being 100 ml.

† Greenhouse incorporation, 3 weeks.

‡ Pennsylvania station plat 23.

‖ By digestion with 1.115 HCl and deduction of mineral components.

§ Crystalline normal carbonate, 4 years' exposure in lysimeters.

UTILITY OF BOILING AMMONIUM CHLORIDE EXTRACTIONS FOR THE DIRECT DETERMINATION OF TOTAL SOLUBLE MATTER IN HIGHLY CALCAREOUS AND SALINE SOILS

It seemed probable that the procedure could be utilized advantageously with the soils of certain lysimeter experiments in which unusually heavy additions of either limestone or magnesic materials had been made. Certain of these enriched soils are deemed by the authors to be more difficult than most natural soils, either residual or transported. The chemical analyses of these enriched soils could not be expressed on the basis of the original soil, so great was the mechanical dilution caused by the substantial unabsorbed fractions of the added carbonates. In previous attempts to draw comparisons a series of analyses, such as carbonate CO_2 and acid-soluble calcium, magnesium, iron, and alumina, had been made. These determinations were supplemented and

amplified by computations, but the results were not satisfactory. In those cases where additions of magnesium oxide or carbonate had been made, the variable extent of the transition of added oxide to carbonate and the uncertainty as to degree of hydration of the residual carbonate militated against such computations. For example, assume an attempt to determine the organic-matter changes induced over a long period by a heavy magnesic treatment where the carbon content of the control soil was 2.20 per cent and that of the magnesia-augmented soil was 2.0 per cent. The apparent loss of 0.2 per cent, or 10 per cent decrease, below the original organic carbon content of the soil would not be an actual loss, since the soil had been diluted by the heavy addition of magnesia. Again, if carbonate CO_2 is found to be 4.30 per cent, then on the basis of a postulated occurrence of $MgCO_3$, the residual carbonate would be 7.23 per cent of the mixture; on the basis of $MgCO_3 \cdot 3H_2O$ occurrence, the value would be 13.52 per cent of the mixture; on the basis of $3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$ occurrence, the value would be 11.90 per cent of the mixture. Hence, in the computations for residual CO_2 in possible magnesium carbonate variables, the correction would cover a range between 7.23 per cent and 13.52 per cent of the enhanced weights of the mixtures of the original soil and magnesic additions. This is sufficient to vary the carbon computations between a minus value and a plus value, in one case an indication of a slight loss of carbon, in the other, a decided increase of from 2.16 to 2.31 per cent of carbon.

There was also a considerable absorption of base by the soil—magnesium, absorption greatly exceeding that of calcium—which called for acid-extraction and analysis and additional corrections. It therefore seemed that, for the purpose of any single analytical determination such as that for the organic carbon or that for the nitrogen of heavily limed lysimeter soils, the simple extraction with boiling ammonium chloride should assure comparable residues of a specific soil, limed and unlimed, as the basis for computations of analytical data.

To throw light on this point, the three lysimeter soils of table 6 were studied —an unlimed clay loam, the same loam treated 10 years previously with $CaCO_3$, and the same loam likewise treated with magnesium hydroxy-carbonate. A 10-gm. charge of each soil, oven-dry basis, was placed in a 600-ml. Pyrex beaker, and 100 ml. of *N* NH_4Cl was added, together with 200 ml. of water. The beaker was covered with a watchglass and heated to vigorous boiling over a Bunsen burner. For this acid soil, and also for all acid soils studied, it was found that the boiling need be continued only until 200 ml. of water is driven off. But for those soils that contained large residues of either lime or magnesia, an additional 100-ml. volume of water was added and boiled off. In all cases the volumes in the covered beakers were replenished to prevent diminution below 100 ml. Complete disintegrations were obtained by boiling for 1 hour after the vapor ceased to give indication of ammonia. This was tested by placing a strip of moistened neutral litmus paper in the lip of the beaker

and comparing the color of this paper with that of a moistened blank. For dolomitic soils the more sensitive brom cresol blue test, given later, should be used.

After cooling, each mixture was transferred to a 56-mm. Büchner with *N* NH_4Cl and washed therewith through a *hardened* filter to a volume of 250 ml.; the clear filtrate was analyzed later for Ca, Mg, Fe_2O_3, Al_2O_3, and Mn_3O_4. The residue from the ammonium chloride washings was washed with 95 per cent ethyl alcohol until free of Cl ions and sucked dry; the alcohol required usually amounted to 300–400 ml. The residue was then policed with water into a porcelain evaporation dish of slightly larger diameter than the outside diameter of the Büchner, evaporated to a small volume on a steam bath, transferred to a small weighed platinum dish where the evaporation was completed. The residue was then dried in an oven at 105°C. for 2 hours, cooled in a desiccator for 30 minutes, and weighed rapidly to obviate gain in moisture.

The detailed analytical results for the limed soil of table 6 are expressed in percentage of the extracted oven-dried residues, whereas the data for the control soil are given on its original basis. The total solvent action exerted on the soil by this boiling and extraction procedure is shown in the lower space of table 6.

In computing the analytical values found for CO_2, CaO, MgO, etc., it was necessary to postulate the degree of hydration and carbonation of the residual magnesia. The computations were made on the assumption that, under the partial CO_2 pressures that prevail in the soil system, the most stable composition of the residual magnesia is the form $3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$. It was difficult to prove the correctness of this assumption, which was used in reporting the outgo of magnesium (7) and in a study of the state of the non-carbonate residues of magnesium by the use of dilute acid extractions (8). Further supporting evidence is now found, however, in the close agreement shown for the sum of the hypothetical combinations and the actual loss on extraction, as registered by the bottom line of table 6.

On the other hand, if it be postulated that the residual magnesium is in the form of $MgCO_3 \cdot 3H_2O$, then the computed sums of the several components dissolved would be 16.51 per cent of the soil residue, or 3.46 per cent more than the actual determination of 13.05 per cent. Hence, after the complete removal of the accumulated fixed bases and residual carbonates by a boiling solution of ammonium chloride and supplemental leachates, the weighed residue from the magnesia-treated soil was thus found to be comparable with the residue of its control soil that had been subjected to the same analytical manipulation.

The carbonate residues from the added calcium carbonate occur in the original chemical form, since here no question of carbonate hydration is involved; hence, the data of the fourth column of table 6 do not require the hypothetical assumptions used in the computations that were applied to the

magnesia treatments. The concordance between the sum of the dissolved components and the direct loss on extraction from the calcareous soil demonstrates that the boiling ammonium chloride extraction and leaching is an accurate technic for the joint extraction of the residual calcium carbonate and the exchangeable calcium content of heavily limed soils.

A point to be emphasized is that the data also show that the boiling ammonium chloride exerts a meager solvent action on the other components of a calcareous soil. This solvent action was materially less than that found by the use of any known method that is capable of extracting all exchangeable bases. From the foregoing data and discussions it is evident that the pre-

TABLE 6

The actual loss of weight of unlimed, $CaCO_3$-treated, and $MgCO_3$-treated soils by the ammonium-chloride digestion-extraction procedure, in comparison with the summation of the separate components removed

DISSOLVED COMPONENTS	UNLIMED SOIL	$CaCO_3$-TREATED SOIL	$MgCO_3$-TREATED SOIL*
	per cent	*per cent*	*per cent*
1. Total calcium, CaO	(0.064)	(9.520)	(0.220)
2. Total magnesium, MgO	(0.013)	(0.065)	(5.760)
3. Carbonate CO_2	0	7.010	4.300
4. CaO or† MgO equivalence of CO_2	0	8.920	5.250
5. Absorbed calcium, CaO	0.064	0.600	0.220
6. Absorbed magnesium, MgO	0.013	0.065	0.510
7. H_2O due to residual† $MgCO_3$	.....	.....	2.350
8. H_2O due to hydration of absorbed CaO and MgO	0.026	0.221	0.301
9. Mn_2O_4	0.092	0.092	0.092
10. $Fe_2O_3 + Al_2O_3$	0.015	0.015	0.015
11. Summation of unbracketed data	0.210	16.92	13.04
12. Loss on extraction	0.310	17.07	13.05
13. Deficiency by summation of separate components	0.100	0.15	0.01

* Additions of precipitated hydroxy carbonate.

† Computed on the basis of CO_2 content as in $3MgCO_3 \cdot Mg(OH)_2 \cdot 3H_2O$.

scribed boiling of a soil in an ammonium chloride solution and attendant washing offers a rapid and accurate procedure for the extraction of *absorbed bases and their carbonates in one operation*, with nugatory effect upon the acidoid residue and minerals.

Fineness of Soil Samples.—In using the subsequent procedures, it is recommended that the entire sample of ordinary soils be ground to pass a 0.5-mm. sieve. For soils of high content of either limestone or dolomite, grind to pass

a 0.25-mm. sieve. This fineness is not essential for complete carbonate decomposition, but is intended to insure a homogeneous sample.

AMMONIUM DISTILLATION PROCEDURE FOR THE RAPID DETERMINATION OF TOTAL ABSORBED BASES,[3] REGARDLESS OF NEUTRAL SALTS

In this paper it has been shown that the ammonia liberated from the digestion of soils with ammonium chloride is due in part to the decomposition of carbonates in the soil and in part to the displacement of the absorbed bases, primarily calcium and magnesium. But it was also noted that the liberated ammonia was not in exact equivalence to the sum of the carbonates and the absorbed calcium and magnesium, because of the fact that varying quantities of ammonia withstood the boiling and were retained by the soil. This finding can be used advantageously to determine accurately the total quantities of absorbed bases, regardless of the presence of carbonates, without a preliminary aqueous extraction of salts—since these do not vitiate the results—and without direct determination of the several bases present in the NH_4Cl extract.

The technic is as follows: Introduce into a 500-ml. distillation flask (preferably Pyrex unit 1370), 10 gm. of soil, 100 ml. of neutral normal ammonium chloride, and 200 ml. of ammonia-free distilled water. Boil the suspension and collect the distillate in standardized acid until a volume of 200 ml. is obtained. For acid soils in general this volume of distillate is sufficient. For unknown soils and those of high carbonate content a larger distillate may be required. To insure that complete reaction and distillation have taken place, 100 ml. of water is added and recovered by distillation. Repeat this step until 100-ml. fractions show a titration value of 1 ml. or less of 0.1 normality, at which point the small evolution of ammonia is attributable solely to the exceedingly slow release of the NH_4 ions retained by the soil.

Transfer the suspension to a 56-mm. Büchner filter and wash with unaltered 95 per cent ethyl alcohol until free of ammonium chloride. Return the ammonium-chloride-free residue to a distillation flask, previously washed free of any ammonium salts; add an aqueous suspension of 0.5 gm. of MgO; and distill 200 ml. into 0.1 normal acid, 5–10 ml. being usually required. Combine this titration value with that obtained from the ammonium chloride distillation, and express the combined value as milliequivalents, each ml. of 0.1 normality ammonia-yield representing 1 m.e. per 100 gm. of soil. From this value subtract the separately determined carbonate-CO_2 value (12) and the ammonia recovered in the first 50-ml. distillate of the blank NH_4Cl solution to obtain the milliequivalents of total absorbed bases.

Note.—The ammonium chloride distillations should be carried out in apparatus not previously used for Kjeldahl distillations, unless particular precautions are taken to insure complete absence of alkali from flask, stopper, and also glass trap.

[3] Hissink's *S* value.

BOILING AMMONIUM CHLORIDE DIGESTIONS FOR THE DETERMINATION OF ABSORBED AND EXCHANGEABLE CALCIUM, MAGNESIUM (8), SODIUM, AND POTASSIUM, REGARDLESS OF CARBONATES

It has been recognized that the presence of $CaCO_3$ in a soil precludes the use of ammonium chloride in the determination of exchangeable calcium, because of the solvent action of the chloride on $CaCO_3$. Hissink (4) therefore suggested that NaCl be used and that supplemental extraction with NH_4Cl be carried out to determine exchangeable magnesium, sodium, and potassium. The latter procedure requires, of course, the precise elimination of calcium, just as though a quantitative determination of that base were to be made. Recently, Chapman and Kelley (1) suggested the use of 0.2 *N* KCl in a 68 per cent alcoholic solution for the determination of exchangeable calcium in calcareous soils. It is obvious that, as in the use of the Hissink method, a supplemental extraction with ammonium chloride is required for the determination of the exchangeable sodium and potassium.

The ammonium chloride digestion procedure is carried out as follows: Introduce a 10-gm. charge of soil into 100 ml. of normal ammonium chloride in a 600-ml. beaker; add 200 ml. of water; cover the beaker and boil the volume of the suspension to 100 ml. Replace the vaporized water in 100-ml. fractions, as in the distillation procedure, until a moistened strip of *high-grade neutral* litmus paper shows no indication of ammonia when exposed to the vapors passing from the lip, a control moistened strip being used for comparison. After absence of NH_3 is indicated, boil off 200 ml. additional. An effective digestion and end-point control is provided by the following brom cresol blue test.

The brom cresol blue test for indicating NH_4Cl-soil digestion end-point.—Because of the variable quality of the litmus paper on the market it was found desirable to introduce a more sensitive test to indicate the end-point of decomposition of soil carbonates and base replacement in the covered-beaker digestion procedure. After tests with a number of widely divergent soils the following dependable end-point test was adopted. Prepare a 0.04 per cent solution of brom cresol blue by grinding 0.040 gm. of the dye in an agate mortar with 25 ml. ethyl alcohol; dilute with water; add 5 ml. of 0.1 *N* HCl and make to 100 ml. This gives a 0.04 per cent brom cresol blue solution in 0.0005 *N* HCl. Dip a slender stirring rod into this indicator solution; remove hanging drops and apply the end of the moistened rod to a 15 x 30 mm. strip of heavy non-acid washed filter paper. This should make a distinct yellow spot of about 7–8 mm. diameter surrounded by a pale blue ring. With tweezers, hold the test paper to the spout of the covered beaker of the boiling NH_4Cl-soil suspension so that the steam strikes the yellow spot. If the yellow spot fails to change completely to a solid blue within 6–8 seconds, the digestion is complete. The filtered solution will then have a pH of 4.4 $\pm$0.2.

Filter, wash with eight 20-ml. washings of ammonium chloride, and make to

volume for the determination of the desired bases. If the soil contained calcium carbonate, the calcium in that combination can be corrected for by subtraction of the calcium equivalence of the CO_2 separately determined by use of a deoxidant (12). A similar correction can be applied for carbonate occurrences of magnesium in soils previously treated with magnesic materials. If the soil is a transported soil and is known to contain dolomite, and a high carbonate occurrence is paralleled by unusually large quantities of dissolved magnesium, the magnesium determination can be corrected by assigning thereto one half of the carbonate equivalence, the other half being applied to the calcium. This last procedure is not ideal, since the 1 to 1 ratio is not a constant for dolomites, but affords an approach to the proximate determination of exchangeable calcium and magnesium in those soils that contain residues of undisintegrated dolomite.

BOILING AMMONIUM CHLORIDE DIGESTION PROCEDURE FOR THE DETERMINATION OF BASE-EXCHANGE CAPACITY, REGARDLESS OF CARBONATES

To assure the subsequent saturation with ammonium chloride that is required for determination of base-exchange capacity in calcareous soils, it is essential to effect complete displacement of absorbed bases and removal of carbonates. It has already been shown that this result is accomplished by the boiling digestion with ammonium chloride according to the following procedure:

Boil the suspension vigorously, as directed for determination of absorbed and exchangeable bases. Remove from source of heat, *cool*, add 15 ml. of 0.1 *N* NH_4OH[4] to the suspension while stirring, and allow to stand at room temperature at least 1 hour. Then wash the soil residue on a 56-mm. Büchner funnel with neutral normal ammonium chloride, taking care to police all of the soil from the beaker and the cover glass. Wash the soil residue on the filter 10 times with 20 ml. *N* NH_4Cl per washing. Continue to wash on the same filter with neutral ethyl alcohol until the washings show no trace of Cl ions, taking care to wash any NH_4Cl that may creep up to the edge of the funnel. Transfer the alcohol-washed soil and its filter to a distillation flask, add 0.5 gm. of MgO in aqueous suspension and distill off the absorbed NH_4. On the basis of a 10-gm. charge of soil, each ml. of 0.1 normal ammonia in the distillate represents 1 m.e. of base-exchange capacity per 100 gm. of soil.

BOILING AMMONIUM CHLORIDE PROCEDURE FOR THE PREPARATION OF SOIL FOR MECHANICAL ANALYSIS

The International Method for the Mechanical Analysis of Soils prescribes that the soil be subjected to a preliminary digestion with 0.2 *N* HCl to decompose carbonates. According to Russell (11), this preliminary treatment may cause a loss of as much as 2 to 3 per cent of dissolved sesquioxides and silica.

[4] Empirically determined as the optimal to effect speedy neutralization of the built-up absorbed hydrogen and to minimize volume of ammonium chloride required to attain a neutral leachate.

To eliminate this difficulty, we advocate that the soil be digested with a boiling normal solution of ammonium chloride, as prescribed by us in the beaker-digestion method for the determination of total dissolved bases. As pointed out, this preliminary digestion effectively eliminates all of the carbonates and neutral salts without appreciable effect upon the sesquioxides or the silica of the soil. The actual loss in weight suffered by the analytical charge through the solvent action of the ammonium chloride is readily determined: Subtract the weight of the alcohol-washed and dried soil residue from the initial moisture-free soil weight. The mechanical analysis can then be carried out on the modified charge.

SUMMARY AND CONCLUSIONS

A study was made of the activity of boiling neutral normal ammonium chloride solution upon soils rich in carbonates and of high absorbed calcium and magnesium content.

The speed and completeness of the disintegration of limestone and of dolomite of different degrees of fineness, as measured by liberated ammonia in the absence of soil, was determined in a preliminary study.

The ammonia-liberations from soil suspensions in boiling neutral ammonium chloride were found to be caused, not only by carbonates, but also by absorbed bases, which Di Gléria had concluded were not affected by the ammonium chloride.

The ammonia-liberations were found to be closely approximate to the joint values of the carbonates decomposed and the absorbed bases replaced; but the total of dissolved bases could not be expressed as the absolute equivalence of the ammonia distilled from the ammonium chloride, because of the variation that soils show in their specific capacities to retain NH_4 ions. Absolute equivalence was attained, however, when the NH_4 held by the alcohol-washed and NH_4Cl-free soil was liberated by boiling with MgO and the ammonia so recovered was added to that obtained in the distillation with neutral normal ammonium chloride.

It was found that boiling ammonium chloride affected *complete* disintegration of the several types of carbonate and *complete* replacement of absorbed and exchangeable bases.

The method has the advantage of speed and requires only a small fraction of the ammonium chloride utilized by other methods.

In effecting complete removal of bases, the ammonium chloride exerted only a nugatory solvent action upon the sesquioxides and silica in both humid and saline soils.

Regardless of the initial soil reactions, pH 4—pH 10, the final pH values of the digestions were within the pH range of 4.4 to 4.6.

The alternative procedures: (*a*) quantitative distillation of ammonium and the supplementary CO_2 determinations, and (*b*) the beaker digestion for the

determination of the several bases, can be utilized in soil research with soil samples of such fineness as to insure uniform distribution of the carbonates:

A technic is prescribed for the determination of absorbed Ca, Mg, Na, and K by a single extraction, regardless of carbonates.

Total absorbed bases can be determined as the equivalent sums of the two values: (*a*) the ammonia evolved from the boiling soil suspension in neutral ammonium chloride and (*b*) the NH_4 ions retained by the soil and subsequently released by boiling with MgO, regardless of occurrences of soluble neutral salts.

Total bases can be determined in those soils that do not yield their absorbed magnesium content by protracted cold extractions with either dilute acids or cold ammonium chloride.

Experimental soils that are "diluted" with large residues of carbonates can be restored to a condition comparable to that of the same soil untreated, and thus admit comparisons otherwise not feasible.

Total base-exchange capacity can be determined subsequent to the boiling with ammonium chloride for the elimination of carbonates, by neutralization of the acid-reacting suspension and leaching of the soil residue with neutral ammonium chloride, removing the solute NH_4Cl, adding MgO, and distilling the absorbed NH_4.

The boiling ammonium chloride extraction can be used for the digestion of carbonate-impregnated soils prior to their mechanical analysis, in lieu of, and as a decided improvement upon, preliminary digestion with 0.2 *N* HCl as now prescribed in the international procedure.

REFERENCES

(1) Chapman, H. D., and Kelley, W. P. 1930 The determination of replaceable bases and the base exchange capacity of soils. *Soil Sci.* 30: 391.

(2) Di Gléria, J. 1929 Die Bestimmung der austaschfähigen Basen in der Böden. *Trans. Internatl. Soc. Soil Sci., Comm.* II, A: 64–68.

(3) Gedroiz, K. K. 1924 The determination of the cations found absorbed in soils by the hydrochloric acid method. *Soil Sci.* 16: 473–474.

(4) Hissink, D. J. 1923 Method for estimating absorbed bases in soils and the importance of these bases in soil economy. *Soil Sci.* 15: 269.

(5) Kelley, W. P., and Brown, S. M. 1924 Replaceable bases in soils. Calif. Agr. Exp. Sta. Tech. Paper 15.

(6) MacIntire, W. H., and Shaw, W. M. 1925 The disintegration of limestone and dolomite separates as influenced by zone of incorporation. *Soil Sci.* 20: 403–416.

(7) MacIntire, W. H., and Shaw, W. M. 1930 Normal magnesium carbonate in comparison with "light" and "heavy" oxides and carbonates in the soil. *Jour. Amer. Soc. Agron.* 22: 919–934.

(8) MacIntire, W. H., Shaw, W. M., and Robinson, B. 1934 The distinction between magnesium absorbed and that exchangeable, four years after lysimeter incorporations of oxides and carbonates. *Soil Sci.* 37: 289–304.

(9) MacIntire, W. H., Shaw, W. M., Robinson, B., and Sanders, K. B. 1933 The effects of additions of certain Colorado soils upon the outgo of bases, chlorides, and sulfates from a Tennessee soil. *Soil Sci.* 36: 437.

(10) MacIntire, W. H., and Willis, L. G. 1915 The determination of soil carbonates—a modification. Jour. Ind. & Eng. Chem. 7: 227.

(11) Russell, E. J. 1932 Soil Conditions and Plant Growth. Longmans, Green & Co.

(12) Shaw, W. M. 1931 Determination of carbon dioxide in soil carbonates—A modification of the official method. *Jour. Assoc. Off. Agr. Chem.* 14: 283–292.

COLLOID CHEMICAL ASPECTS OF CLAY PAN FORMATION IN SOIL PROFILES

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Numerous soil types in the central part of the United States are characterized by heavy clay pans, i.e., accumulations of colloidal clay particles in the B-horizon of the soil profile. The pans form in loess under a cover of prairie or timber vegetation and are most conspicuous in areas of poor drainage. Typical clay pans such as those in Putnam silt loam cause serious handicaps to agricultural practices. In the springtime the surface water remains stagnant and seed-bed preparation is delayed. In general, plant root development is stunted and tree growing is almost impossible.

Figure 1 shows the distribution of clay particles less than 1μ (0.001 mm.) in diameter in a poorly drained and in a well-drained profile in the State of Illinois (5). It will be noticed that in certain parts of the soil column the clay particles make up 50 per cent of the soil weight.

Generally speaking, the origin of clay pans may be due to several causes: first, by deposition of colloids in water (geological process); secondly, by local formation of clay *in situ* by intensive weathering; and thirdly, by translocation and subsequent deposition of clay particles within the soil profile. This last type of pan we shall designate as *illuvial pan*, and all subsequent discussions will be restricted to this specific variety.

NATURE OF THE COLLOIDAL PARTICLES ISOLATED FROM ILLUVIAL CLAY PANS

Analyses of clay extracted from the various horizons of clay pan profiles indicate that the properties of the colloids vary but little, and one is forced to conclude that the clay particles migrate as a whole rather than in the form of colloidal SiO_2, Al_2O_3, and Fe_2O_3 with subsequent recombination. The particles isolated from the Putnam clay pan exhibit pronounced streaming double refraction, which proves that they are not of spherical shape. The X-ray patterns are very distinct and resemble those of montmorillonite.[1] The silica-alumina ratio fluctuates between 3.0 and 3.2, and that of the silica-sesquioxide ratio, between 2.5 and 2.8. The base-exchange capacity amounts to 60–70 m.e. per 100 gm. colloid, depending on the method of determination.

The constancy of the composition of the colloidal clay particles throughout

[1] HOFMANN, U., AND ENDELL, K. X-ray analyses of Putnam clay. Unpublished data.

the profile justifies a study of their behavior in artificial sand columns under controlled laboratory conditions. Such investigations might be called experimental soil profile formation, and the results should prove to be of value in the interpretation of natural soil systems.

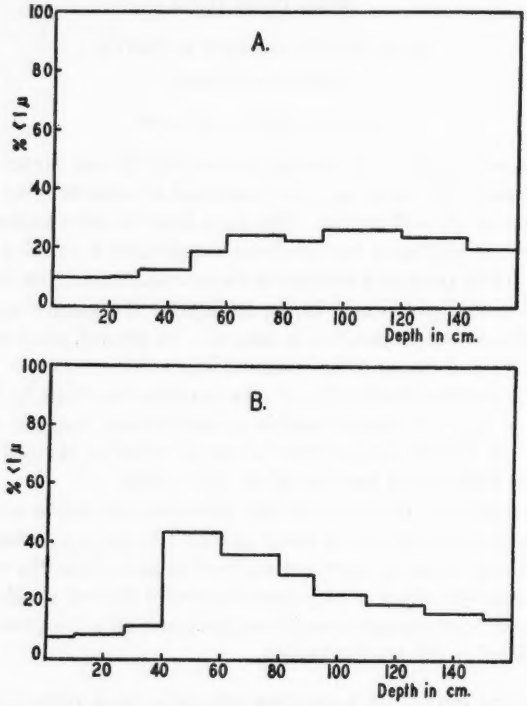

FIG. 1. ACCUMULATIONS OF CLAY PARTICLES LESS THAN 1μ IN DIAMETER IN VARIOUS HORIZONS OF A WELL-DRAINED (A) AND A POORLY DRAINED (B) PROFILE

Ordinate indicates per cent clay per 100 gm. of soil

EXPERIMENTAL PROCEDURE

Since a detailed description of the experimental part is to be reported elsewhere, only the most essential points will be mentioned here. In principle, a series of artificial soil skeletons, composed of quartz grains or spherical glass beads, was prepared, and clay suspensions (sols) were passed through the beds. The effects of various treatments on the velocity of pan formation in these artificial systems were recorded quantitatively.

Soil skeleton.—Carefully purified quartz particles of uniform size (table 1), were packed to a height of 5–10 cm. into burettes of 12 mm. diameter. A standard rate of flow of 5 cc. of distilled water in 23–24 seconds was arbitrarily selected, and the head necessary to give this flow was determined empirically for each sand column.

Preparation of clay sols.—A sol was prepared by adding NaOH to an electrodialyzed Putnam clay at the rate of 60 m.e. of base per 100 gm. of colloid. To insure equilibrium the suspension was shaken at intervals for several weeks and then diluted to a 0.3 per cent sol.

Criterion of pan formation.—When properly treated Na-clay sols were allowed to percolate through the skeleton, the time required for 5 cc. to pass through varied in a regular manner. The change in the rate of flow was selected as the best measure of pan formation. Zero change indicates absence of clay accumulation, whereas a decreasing rate of flow corresponds to pan development. The results are reported as relative percolation velocities, calculated as follows: The time required to deliver the first 5 cc. of sol (t_1) was taken as unity. The time used by each successive aliquot (t_n) was then divided into that of the first 5 cc. ($t_1:t_n$). Thus, when the time had doubled, the relative rate of flow or percolation velocity would be 0.5. In the graphs the fine dotted lines pass through the observational values, while the heavy lines represent the fitted trend.

INHERENT DISPERSION TENDENCY OF COLLOIDAL CLAY SYSTEMS

In absence of flocculation agencies the Putnam clay particles have a natural tendency to "disperse." Air-dry chunks of colloidal clay submerged in distilled water will hydrate and swell, and the particles will separate from one another. Although the Brownian movement is not intense enough to cause much diffusion and dispersion, it is important to note that the colloidal particles are free to glide easily along one another. Mere convection currents and minor disturbances, such as gentle shaking, cause the system to disperse sufficiently to form a temporary, or even a permanent, sol. Thus, Putnam clay in contact with water and not subjected to mechanical disturbance forms neither a sol nor a true gel, but a so-called *coacervate*, i.e. a gel-like system in which the particles possess individual mobility (2). This concept is essential for a clear understanding of the behavior of clay particles in soils.

When a fine clay sol is passed through beds of coarse quartz sand or glass beads, no pan formation occurs and, furthermore, the clay particles do not adhere to the skeleton except for negligible surface tension effects. Complete separation of quartz sand and colloidal clay by mere washing with distilled water is always possible. As a matter of fact, such a behavior would be expected on theoretical grounds, since both sand and clay are negatively charged and, consequently, repel each other.

Observations of the sort mentioned strongly suggest that under natural field conditions colloidal clay particles of the Putnam clay type tend to "disperse" and migrate with the water currents unless held back by some special mechanism.

THE SIEVE ACTION PRINCIPLE IN PAN FORMATION

In a soil profile the colloidal particles can migrate only in the pore channels of the soil skeleton. If the diameter of the smallest pore exceeds the greatest diameter of a clay particle, the latter will freely follow the percolating water and no tendency of pan formation will exist. In any other case partial or total accumulation of colloids is certain to occur. Generally it can be said that a soil skeleton can filter out or retain colloidal particles only if the pores themselves are of ultramicroscopic dimensions. Spherical quartz grains would need to possess radii of 0.2μ (order of magnitude), in other words, they would have to be clay particles also, though of somewhat larger size. Angular quartz

grains could be of macroscopic size, silt for instance, and still produce channels with occasional colloidal cross-sections. Evidently the *formation of clay pans by sieving out the colloids mechanically is restricted to very fine-textured soil skeletons.*

TABLE 1

Particle and pore size of soil skeletons (on the basis of spherical shape and close packing)

PARTICLE GROUP			DIAMETER OF PORES*
Conventional name	Taylor sieve designation	Diameter of grains	
	mesh	*mm.*	*mm.*
Beads	.....	0.56–0.05	0.087
Coarse sand	20– 40	0.83–0.39	0.129–0.054
Medium sand	40– 60	0.39–0.25	0.054–0.038
Fine sand	60– 80	0.25–0.18	0.038–0.027
	80–100	0.18–0.15	0.027–0.023
	100–150	0.15–0.10	0.023–0.016
Very fine sand	150–300	0.10–0.05	0.016–0.008
Coarse silt	.....	0.05–0.02	0.008–0.003

*From Slichter (6).

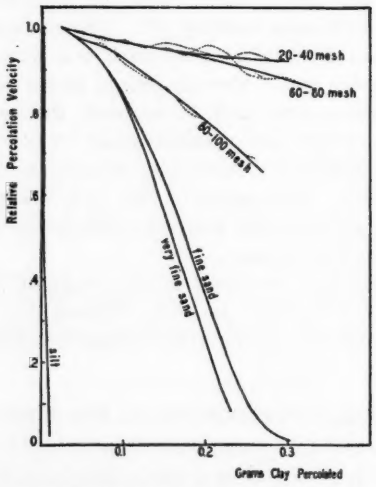

FIG. 2. THE SIEVE ACTION PRINCIPLE OF CLAY ACCUMULATION

Passage of a heterogeneous Na-clay sol through columns of coarse and fine quartz grains

Figure 2 illustrates instructively the sieve action principle of pan formation. Na-clay sols were passed through various sand beds, each consisting of quartz particles of different, but uniform, size. It is clearly seen that the smaller the average diameter of the grain the more rapid is the pan formation as indicated

by the reduction of the percolation velocity of the sol. Sand of 20–40 mesh caliber acts very slowly, whereas silt appears to be very effective. In order to explain fully the sequence of the curves it should be pointed out that the sol used contained, not only colloidal particles, but also larger ones. In fact, occasionally aggregates of microscopic size were found floating in the sol (bacterial colonies). The coarse sand is able to retain the large aggregates, and the rate of flow is slightly reduced. As the size of the quartz grains decreases, coarse clay particles are caught, which help to reduce the effective cross-section of the pores until the latter reach colloidal dimensions and almost stop the passage of the sol.

Besides using a specific clay sol and a variety of sand beds we percolated various clay sols through beds of equal particle size. For this purpose the original sol was fractionated by filtering it with a column of 60–80 mesh sand,

TABLE 2

Effect of mechanical fractionation of a clay sol on the velocity of pan formation in a fine sand bed

SUSPENSION PERCOLATED	RELATIVE PERCOLATION VELOCITY	
	Original sol	Finer fraction
First 5 cc. sol	1.000	1.000
Second aliquot	0.958	0.995
Third aliquot	0.914	0.995
Fourth aliquot	0.835	0.989
Fifth aliquot	0.742	0.966
Sixth aliquot	0.635	0.976
Seventh aliquot	0.509	0.953
Eighth aliquot	0.346	0.966
Ninth aliquot	0.238	0.961
Tenth aliquot	0.107	0.963

which removed the coarse particle group (microörganisms). Subsequently, both the original sol and the refined part (condensed to a 0.3 per cent suspension) were subjected to pan formation processes in beds of fine sand. According to table 2 the resulting differences are very great. The original sol develops a pan much more rapidly than the finer fraction.

In a natural soil both the skeleton and the mechanical composition of the clay group are heterogeneous. There will always be enough pores of sufficiently small size to retain coarse primary particles, but unless the texture is very heavy the truly colloidal clay will not tend to accumulate much within the profile.

EFFECT OF ELECTROLYTES ON PAN FORMATION

Sieve action by sand or silt skeletons affects mainly the large clay particles. If one were able to increase the size of the colloids, pan formation would likely

be enhanced. One of the most effective means to bring about aggregation in a colloidal system consists in the introduction of electrolytes. Since clay particles are negatively charged the dominating influence will be exerted by the positive ion (cation) of the electrolyte. Table 4 contains the flocculation values of a number of electrolytes expressed in terms of symmetry concentrations (S).[2] According to table 4 both the charge and the size of the cation are influential, though the former exceeds the latter in effectiveness.

TABLE 3

Flocculation of Putnam clay with chlorides

(Flocculation value expressed in terms of symmetry concentration)

FLOCCULATION CATION			FLOCCULATION VALUE NH_4-CLAY
Symbol	Valence	Size in Å.U.	
Na	1	0.98	7.0 *S*
Li	1	0.78	6.5 *S*
K	1	1.33	5.0 *S*
NH_4	1	1.45	4.5 *S*
Rb	1	1.49	.....
Cs	1	1.65	2.6 *S*
H	1	....	0.87 *S*
Mg	2	0.78	1.1 *S*
Ca	2	1.06	1.1 *S*
Sr	2	1.27	1.1 *S*
Ba	2	1.43	1.1 *S*
La	3	1.22	0.90 *S*
Th	4	1.10	0.75 *S*

TABLE 4

Salts used for pan formation with Na-clay

ELECTROLYTE	VALENCE OF CATION	FLOCCULATION VALUE	FLOCCULATION CONCENTRATION
NaCl	1	17.5 *S*	1 *F*
$CaCl_2$	2	1.4 *S*	1 *F*
$La(NO)_3$	3	1.0 *S*	1 *F*
$ThCl_4$	4	0.85 *S*	1 *F*

The rôle of electrolytes in pan formation was studied for four different salts (table 4) which furnish cations of various charges. The electrolyte concentrations added to the clay systems (before their passage through sand) are expressed in terms of flocculation values, *F*, a procedure which facilitates graphical representation as well as interpretation of the results. The way

[2] The value of $S = 7$ for NaCl means that for a given set of conditions at least 7 times *S* milliequivalents of NaCl must be added to produce flocculation. The letter *S* represents the number of adsorbed (exchangeable) ions in the system under consideration.

in which increasing salt concentration changes the rate of flow is demonstrated in figure 3. To permit comparative analysis between the various electrolytes, figure 4 has been constructed, which forcefully brings out the pronounced effect of the charge of the cation. *The higher the charge the more rapid the reduction of the percolation velocity of the sol.* From the viewpoint of clay pan formation in nature, the difference between Ca and Na is particularly significant, since field observations indicate that many clay horizons are associated with lime concretions.

Special experiments on the flocculating power of $CaCO_3$ and $CaSO_4$ furnished conclusive evidence that both salts, in the presence of their solid phase,

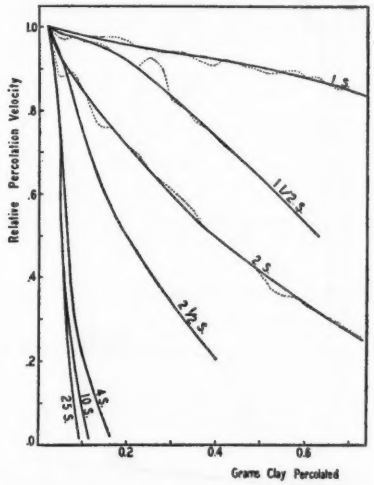

FIG. 3. EFFECT OF ADDITIONS OF $CaCl_2$ (SYMMETRY CONCENTRATIONS) ON THE RATE OF PAN FORMATION

Abscissa: grams of clay as 0.3 per cent sol percolated through the sand column; ordinate: rate of pan formation which is inversely proportional to the relative percolation velocity of the clay sol.

coagulate the clay systems, no matter what the nature of the adsorbed cation (H, Na, K, NH_4, Ca, Mg).

Four samples of natural ground water from typical clay pan areas in Missouri and Illinois contained enough electrolyte to flocculate completely natural Putnam clay, even after three-fold dilution with distilled water.

Closer examination of figure 4 reveals the interesting fact that the different behavior of the various electrolytes persists up to concentrations many times the flocculation value. Apparently the cation does more than merely neutralize the charge of the particle; it also causes the formation of characteristic gross aggregates, which vary in size, density, and stability, and which impress their individuality on the kind and speed of pan formation.

Significance of hydration.—The floccules formed are relatively loose aggregates of strongly hydrated primary clay particles. During the passage through the sand the secondary aggregates may collide with quartz grains and temporarily break up into smaller units and thus retard accumulation. One would expect that dehydrating agencies, which remove the water shells and bring the primary particles into contact, would form more stable aggregates and thus accelerate pan formation. Experiments support this concept. Addition of

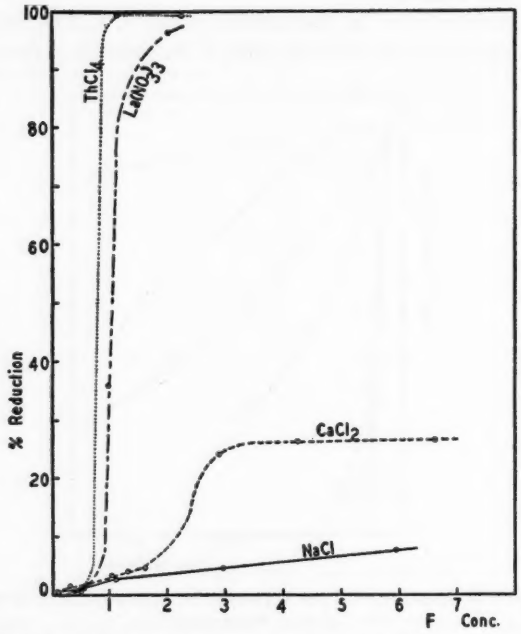

FIG. 4. INFLUENCE OF THE VALENCE OF THE CATION ON THE RATE OF PAN FORMATION

Ordinate: reduction of initial percolation velocity by 0.021 gm. of clay (in form of 0.3 per cent sol); abscissa: electrolyte concentration in terms of flocculation values.

alcohol to Na-clay in the presence of $CaCl_2$ and NaCl enhances the building up of impervious clay horizons in the sand column.

Rôle of protective humus.—Colloidal humus exerts protective action on suspensoids, i.e., it shifts the flocculation value of electrolytes to higher concentrations, as seen in figure 5. To flocculate 5 cc. of neutral Putnam clay sol for a given set of conditions, 0.1 m.e. of NaCl is necessary. Mixing the clay sol with neutral humus extract prior to the addition of electrolyte increases the stability many fold. The action of humus to protect the clay sol from precipitation manifests itself in the rate of pan formation, as indicated in figure 6. Natural colloidal humus from a sample of muck was added to Na-clay

at the rates of 0.017 gm. and 0.033 gm. per gram of clay. Twice the symmetry concentration of $CaCl_2$ was added, and the systems were percolated

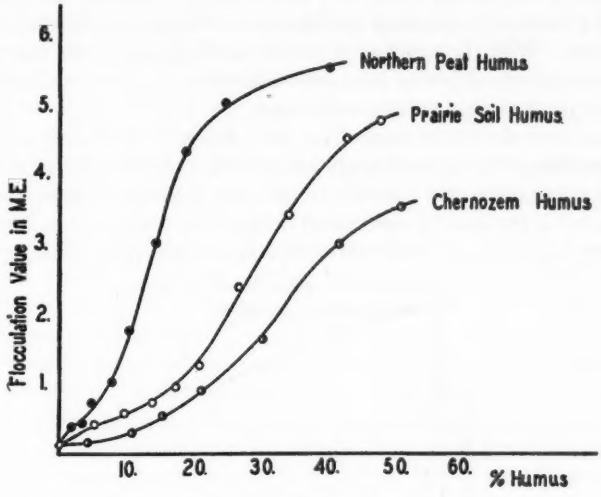

FIG. 5. PROTECTIVE ACTION OF COLLOIDAL HUMUS AGAINST FLOCCULATION OF NATURAL CLAY SOL BY NaCl

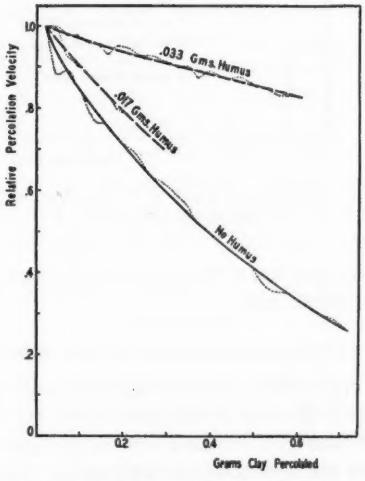

FIG. 6. REPRESSION OF PAN FORMATION BY COLLOIDAL HUMUS

through 20–40 mesh sand. The 0.017-gm. humus sample did not prevent flocculation, yet it retarded considerably the development of the pan. The

other sample fully protected the clay from coagulation and correspondingly prohibited the accumulation of the colloids (fig. 6).

Removal of coagulating salts.—If a pan is being formed in the presence of $CaCl_2$, for example, subsequent percolations of distilled water tend to reverse the process. With the removal of the excess of electrolyte the clay pan disperses and the rate of flow of the solution increases very rapidly; later it slows down and tends to approach the initial state.

Summarizing the rôle of electrolytes, we consider the following points to be most significant: The electrolytes in concentrations near or above the flocculation value cause the clay particles to unite to secondary aggregates, which are retained in the sand by mechanical sieve action. The process is reversible to a very large extent. Colloidal humus favors migration of clay particles.

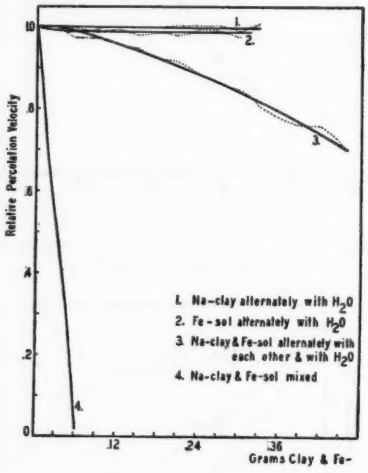

FIG. 7. FORMATION OF ATTRACTION PANS WITH THE AID OF POSITIVE IRON SOLS $[Fe(OH)_3 \cdot x\ H_2O]$

Clay accumulations formed under the influence of coagulating electrolytes we shall designate as *electrolyte pans*.

THE ATTRACTION PHENOMENON IN PAN DEVELOPMENT

If a positive iron sol $[Fe(OH)_3 \cdot xH_2O]$ is passed through a column of spherical glass beads no change in the rate of flow can be detected (fig. 7). However, a thin coat of the Fe-colloid sticks to the beads (or sand grains) and—in contrast to clay particles—can not be washed off with water. Evidently the negative silicate spheres and the positive iron hydroxide particles attract each other and stick together permanently.

A similar attraction phenomenon occurs if positive iron sols and negative clay suspensions are mixed in suitable proportions. Mutual flocculation takes

place and pan formation results at once (curve 4, fig. 7). A fundamental difference exists between this "attraction pan" and the electrolyte pan in that the latter is largely reversible, whereas the former is not. Prolonged leaching with distilled water fails to disperse significantly the illuvial iron-clay horizon.

The two experiments just described can be combined to yield an interesting and unsuspected mode of pan formation. Ten cubic centimeters of 0.3 per cent dialyzed Fe-sol is permitted to pass the skeleton of negative glass beads. The iron hydroxide particles not held by the spheres are leached out with five successive washings of 10 cc. H_2O. Determination of the endoelectrosmotic flow reveals that the entire system has now become electropositively charged. Then 10 cc. of 0.3 per cent Na-clay is percolated through the same bed and the burette again rinsed with water to remove the free particles completely. Electrical measurements show that the entire system has turned negative again. The same process is repeated in the order: iron sol + water + clay sol + water + iron sol + ... etc. The electric charge of the system alternates regularly, always assuming the same sign as the sol that passed through last. Corresponding determinations of the rate of flow of water and sols indicate definitely that a pan is gradually being formed (curve 3, fig. 7). Obviously pan development is due to the building up of alternating layers of iron hydroxide and clay which restrict the width of the pores and reduce the rate of flow of the liquids. Sieve action may finally result.

As in the case of electrolytes, *colloidal humus* protects the sols also from mutual flocculation. If sufficient colloidal humus is added to a clay suspension, addition of Fe-sol may not produce any flocculation. On the other hand, precipitated iron-clay colloids in the form of coarse aggregates readily disperse upon addition of humus (with gentle shaking) to a finely divided, very permanent sol. It is of further significance that colloidal humus reduced the attraction between iron colloids and quartz grains.

The experiments on mutual flocculation lead to the conclusion that in spite of the absence of electrolytes pan formation can take place, even in coarse-textured systems, provided that positive colloids are present. The attraction pans are much more stable than the electrolyte pans.

APPLICATION OF EXPERIMENTAL RESULTS TO THE THEORY OF CLAY PAN FORMATION

The concept of the ideal soil.—Workers engaged in experimental studies of soil formation are handicapped by the lack of soil types the origin of which is completely understood. The authors have found it convenient to think in terms of an *ideal soil profile*, which can be synthesized at will by the operation of but a limited number of soil-forming factors. The parent material of such a soil is supposed to have the following properties: It is made up of uniform spherical particles of fine sand size, and the chemical and mineralogical composition is that of the average values of the igneous rocks. To visualize pan formation in such a system, topography is assumed to be level and the

underdrainage good. Climate will be taken as of the arid-humid transition type on one hand, and of the strictly humid temperate type on the other.

Clay pan formation in the arid-humid transition zone (idealized conditions).—Rain water penetrates the parent material; hydrogen ions of water and CO_2 liberate the bases of the crystal lattices; the minerals break up; and clay particles are formed. The percolating rain water carries the easily soluble substances into the lower part of the profile where they accumulate (carbonate horizon) as a consequence of water absorption, evaporation, and reduced CO_2-pressure. Since rainfall is limited, no water will ever move out of the solum. As soon as the electrolyte concentration in the surface becomes sufficiently low, its clay particles will disperse and move downward with the water currents until they reach a zone where the salt accumulation is high enough to cause flocculation and retention of aggregates by sieve action. Additional clay migrating downward will also be coagulated, or, finding the pore size restricted, filtered out mechanically. In such a soil profile the chemical composition of the clay particles within the various horizons would be expected to be rather homogeneous. It is quite possible that the process just described explains the origin of clay pans in chernozems (1); in other words, they would be called *electrolyte pans.*

Clay pan formation under humid temperate conditions.—According to Norton and Smith (4) the most impervious clay pans in Illinois are found in regions of level topography associated with poor drainage (Cisne silt loam, Putnam silt loam). These clay horizons probably fall into the category of electrolyte pans. They differ from the chernozem phase in general in their more advanced weathering and more pronounced translocation of colloids, and in particular as a result of the fact that flocculation is brought about by the electrolytes of the ground water.

In the absence of temporarily high water tables, clay pan development in humid regions is difficult to understand, unless the attraction phenomenon is taken into consideration. Under conditions of high precipitation the soluble electrolytes are constantly removed by the percolating rain water and the chances for the development of a permanent electrolyte pan are remote. However, the intensified weathering that produces large amounts of clay minerals (3) also favors the formation of positive iron and aluminum hydroxides. Throughout the profile they become attached to the negative quartz grains and, moreover, attract wandering clay colloids, all of which leads toward the development of an attraction pan. In the surface—on account of the protective organic matter—the downward migration of the colloids is enhanced and the differentiation of the soil profile into A- and B-horizons becomes accentuated. In well-drained soils one would not expect very pronounced clay zones, but rather a distribution of the particles over a greater depth. Furthermore, the clay colloids (in contrast to the chernozem pan), would likely vary considerably in their chemical composition, particularly in the silica-sesquioxide ratio.

The modes of pan formation outlined apply to ideal soils, but nature never provides such simple systems. The heavy clay pans observed in temperate regions have undoubtedly developed by combinations and modifications of the "electrolyte" and "attraction" principles.

SUMMARY

For the study of clay pan formation a method has been developed which permits quantitative measurements of clay accumulations in sand columns.

Putnam clay, if sufficiently dispersed, will not form a heavy pan in beds of quartz sand or silt by mere sieve action.

Electrolytes are responsible for the development of "electrolyte pans" as a result of flocculation and retention of coarse clay aggregates. The higher the valency of the electrolyte cation the more rapid is the pan formation. Dehydration enhances clay accumulation, whereas colloidal humus favors translocation.

Positive iron hydroxide sols give rise to "attraction pans" based on adherence of iron hydroxide particles to the negative quartz grains and on mutual coagulation with negative clay colloids.

Principles of clay pan development in ideal soils are discussed.

REFERENCES

(1) Brown, I. C., Rice, T. D., and Byers, H. G. 1933 A study of clay pan soils. U. S. Dept. Agr. Tech. Bul. 399.

(2) Bungenberg de Jong, H. G. 1934 Complex relationships in lyophilic colloidal systems. *Rec. Trav. Chim. Pays-Bas*, 53: 164.

(3) Jenny, H., and Leonard, C. D. 1934 Functional relationships between soil properties and rainfall. *Soil Sci.* 38: 363–381.

(4) Norton, E. A., and Smith, R. S. 1930 The influence of topography on the soil profile. *Jour. Amer. Soc. Agron.* 22: 151–162.

(5) Norton, E. A., and Winters, E. 1930 Pedological aspects of the genesis and morphology of Illinois soils. *Proc. Second Internatl. Cong. Soil Sci.* 5: 91–96.

(6) Slichter, C. S. 1898 A theoretical investigation of the motion of ground waters. *U. S. Geol. Survey Ann. Rpt.* 1898 (part II): 301.

(7) Smith, G. D. 1934 Experimental studies on the development of heavy clay pans in soils. *Missouri Agr. Exp. Sta. Res. Bul.* 210.

SOIL PROFILE STUDIES: VII. THE GLEI PROCESS

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In Russia the popular understanding of glei, according to Vuisotzkii (5), is "a more or less compact, sticky loam or clay parent material, which is not, however, as sticky as the usual loam or clay, frequently with a more or less clearly pronounced light greenish blue tinge."

Glei formation is found in marshes and under conditions of a high water table. The glei horizon is generally water-logged, except when the water table recedes. In this horizon, therefore, anaerobic conditions prevail, which favor reducing reactions and minimize leaching effects. Besides, the ground waters enrich the glei horizon with bases, if such are in solution, and impart to it an alkaline reaction. In the reduction process the ferric iron gives rise to the ferrous iron, and in the case of the hydroxide the weak base changes into the stronger ferrous iron base. Partly because of this fact, therefore, one should expect a higher pH in the glei horizon. Data on the reaction in the glei horizon bear this out. With the receding of the level of the water table, the ferrous iron is oxidized and the pH must naturally drop. This perhaps is the reason why some glei horizons show a lower pH than others.

The simultaneous downward and upward movements of the substances in solution bring about a unique condition with respect to the iron therein contained. The anaerobic state is conducive to reducing reactions and the insoluble iron compounds become soluble and move downward. At the same time the rise of substances in solution by the capillary forces brings back some iron to a point where it might come in contact with the air and precipitate. We thus find an ocherous layer on top of the glei horizon. This is probably the chief factor in the process of bog iron formation. While the upper layer of the glei horizon becomes enriched with iron, the lower layer becomes impoverished of iron. It is in this layer of the glei horizon that we find the mottling effects with the characteristic gray-bluish-green tinge of the material.

In many respects the process of ochre formation is similar to that of the rising of salts in alkali soil formation. This has been pointed out by Vuisotzkii (5), who was probably the first to study glei formation.

Within the podzol zone, especially in its northern belt adjoining the forest tundra, glei formation is not uncommon. This does not exclude its existence in other soil zones, since it is not a climatogenic formation, but a hydrogenic. Indeed Vuisotzkii (5) points out the presence of glei formation in the southeastern Precaspian steppe.

Glei formation has been studied primarily in the podzol zone and has therefore been associated with this zone. Whenever glei formation is found on a podzol soil, it is the C or B horizons that undergo the respective changes. The A_2 horizon remains intact, i.e., the rising waters do not reach this horizon.

Frosterus (1), in his work on the podzols with clay as parent material and a high water table, discusses the question of glei.

An extensive study of soils with a glei horizon in the podzol zone has been made under the direction of the late Glinka by Zavalishin (7).

TABLE 1

Reaction of soils with a glei horizon

(After Zavalishin)

SOIL	HORIZON	DEPTH	REACTION
		cm.	*pH*
Clay marsh	A_0	0– 8	6.2
	A_1	8–18	6.5
	A_2	18–29	6.5
	G	29–42	5.9
	G	42–61	6.5
	G	61–75	7.4
Clay marsh	A_1	0–10	5.8
	A_2	10–25	5.8
	G	25–60	7.3
Clay marsh	A_0	0– 8	5.7
	A_1	8–22	6.2
	G	31–61	6.5
Podzolic-glei	A_1	0–20	5.8
	A_2–G	20–28	6.4
	G	28–40	6.5
Podzolic with a glei horizon	A_1	3–14	5.4
	A_2	14–36	5.8
	B–G	36–56	6.8
	G	56–65	7.0

In table 1, adapted from Zavalishin (7), the reaction of a number of soils with a glei horizon is given. In table 2 the soluble humus and the total humus are recorded.

It will be noted that with depth the soils become less acid and the glei horizon reaches the neutrality point. There is not much organic matter in the glei horizon, but the quantity of soluble humus increases with depth and is higher in the G horizon than in the overlying A horizon, which has a higher total of humus.

An interesting attribute of the glei horizon is the base saturation of its base-

TABLE 2

Water-soluble humus in soils with a glei horizon

(After Zavalishin)

SOIL	HORIZON	TOTAL HUMUS	$KMnO_4$ USED FOR WATER-SOLUBLE HUMUS	SOLUBLE HUMUS
		per cent	*cc.*	*per cent*
Peat podzol; podzolic glei	Humus ortstein (bog-iron)	2.25	2.55	0.00033
Peat podzol; podzolic glei	G (upper portion)	1.35	7.11	0.00093
Glei marsh	G	0.23	5.10	0.00066
Podzol, with ortsand	Humus ortsand	4.19	4.40	0.00057
Peat podzol	Lower portion of humus ortsand	2.16	10.20	0.00132
Peat podzol	G_1	0.51	8.15	0.00105
Peat podzol	G_2	0.41	8.35	0.00108
Peat marsh	G	0.34	7.85	0.00102

TABLE 3

Exchangeable bases in base exchange complex of glei soils

(After Zavalishin)

SOIL	HORIZON	ABSORBED CATIONS			Mg IN TERMS OF Ca	H IN TERMS OF Ca	TOTAL CATIONS IN TERMS OF Ca
		Ca	Mg	H			
		Per cent of dry soil					
Clayey marsh on a heavy loam	A_1	2.15	0.16	None	0.27	.....	2.42
	G_1	0.36	0.11	None	0.16	.....	0.62
	G_2	0.30	0.12	None	0.19	.....	0.49
Podzolic glei sandy loam	A_1	0.12	0.02	0.0006	0.03	0.012	0.16
	A_2	0.05	0.005	None	0.01	.....	0.06
	G	0.07	0.007	None	0.01	.....	0.08
Podzolic soil with a close glei horizon	A_1	0.17	0.04	0.0008	0.06	0.016	0.25
	A_2	0.06	0.02	None	0.03	.....	0.09
	B	0.14	0.06	None	0.10	.....	0.24
	G	0.18	0.10	None	0.17	.....	0.35
Weakly podzolized with a close G horizon on a heavy loam	A_1	0.76	0.23	.....	0.38	.....	1.14
	A_2	0.23	0.04	.....	0.06	.....	0.30
	B–C	0.24	0.12	.....	0.20	.....	0.44
	G	0.24	0.15	.....	0.25	.....	0.49
Weakly podzolic glei loam	A_1	0.38	0.08	.....	0.13	.....	0.51
	A_2–G	0.13	0.05	.....	0.08	.....	0.21
	G	0.18	0.08	.....	0.13	.....	0.31

exchange complex. As indicated in table 3, adapted from Zavalishin, the glei horizon differs but little from the parent material except that it contains slightly less Ca. Its Mg is less mobile than the Ca and in absolute terms the glei contains more Mg than Ca, the reverse of which is true for normal soils. In regions where the ground waters are rich in K and Na, the glei horizon is more sticky and is in a poorer physical condition than an ordinary glei. A case of this nature has been described by Wityn (6), who found the acid and normal

TABLE 4

Al_2O_3 and SiO_2 extracted from glei podzols with 0.05 N HCl and 5 per cent KOH

(After Zavalishin)

SOIL	HORIZON	ON REPLACING THE BASES BY EXTRACTING WITH 0.05 *N* HCl		SOLUBLE IN 5 PER CENT KOH EXTRACT		MOLAR RATIO OF Al_2O_3: SiO_2 FROM KOH EXTRACT
		Al_2O_3	SiO_2	Al_2O_3	SiO_2	
		per cent	*per cent*	*per cent*	*per cent*	
Clayey marsh on a heavy loam	A_1	1.10	Traces			
	G_1	0.40	0.12	0.36	0.44	1:2.09
	G_1	0.46	0.13			
Clayey marsh loam	A_1	0.30	0.028			
	G	0.18	0.025	0.38	0.63	1:2.83
Podzolic glei loam	A_1	0.30	0.012			
	A_2–G	0.16	Traces			
	G	0.16	0.020			
Podzolic glei sandy loam	A_1	0.15	Traces			
	A_2	0.08	Traces			
	G	0.10	0.01			
Podzolic loam with a close G horizon	A_1	0.18	Traces			
	A_2	0.056	Traces			
	B	0.16	0.04			
	G	0.40	0.14			
Weakly podzolized with a close G horizon on a heavy loam	A_1	0.54	....			
	A_2	0.16	Traces			
	B–G	0.30	0.08			
	G	0.38	0.24	0.42	0.81	1:3.1

carbonates in the glei horizon. It is of interest that the horizon overlying the glei horizon is relatively high in absorbed Ca and Mg. This condition is probably due to the capillary rise as the water table rises.

Another interesting attribute of the glei soil is its SiO_2 and Al_2O_3 content soluble in 0.05 *N* HCl (Gedroiz acid) and in 5 per cent KOH solution (Gedroiz method).

From table 4, adopted from Zavalishin, it is clear that the SiO_2 content

extracted with the 0.05 *N* HCl increases with depth. Practically none, however, is found in the filtrate from the podzolized A_2 horizon, notwithstanding the fact that its total SiO_2 content is highest, as compared with the other horizons. The same is almost true for the A_1 horizon. The highest quantity of SiO_2 goes into solution from the glei horizon after the Ca has been replaced. To appreciate more fully the significance of the data, Zavalishin presents figures on the SiO_2 extracted from other soils by the same method:

The B horizon of a sandy podzol from the Leningrad district gave 0.06 per cent of SiO_2; the B horizon of a loam from the same district, 0.03 per cent; an ortsand of a sandy podzol from the Valdai county, 0.01 per cent; and ortstein from Pavlovsk, 0.08 per cent; the B horizon of a red soil from the Bengaza steppe desert, 0.32 per cent; an ortstein of a podzol sandy loam in the Murman region on the river Tuloma, 0.18 per cent.

Leaving out of our discussion the desert soil, for the reason that the soil forming processes are entirely different from those in the podzol zone, we can readily see that, as a rule, the SiO_2 soluble in HCl from the true podzol or podzolic horizons lags far behind that of the glei horizon. Apparently there is some decomposition of the base-exchange complex in the glei horizon. Zavalishin adds that "probably some amorphous SiO_2 is carried to the glei horizon from the upper horizon and from the ground waters."

As to the HCl-soluble alumina, Zavalishin points out that

. . . it is higher than in A_2 and in most cases lower than in A_1. In the B horizon the soluble Al is even higher than in the A_1 horizon.

Turning our attention to the results of the alkali, 5 per cent KOH, extracts [Gedroiz (2) method], it is to be noted that in the soils investigated the molar ratio of Al_2O_3:SiO_2 fluctuates between 1:2.09 and 1:3.8. Thus there is a slight excess of SiO_2. This indicates the probability of splitting off SiO_2 by the action of the ground waters.

One other important feature in the gleiing process is the presence of ferrous iron in the glei horizon. Zavalishin investigated a series of soils and presented his findings in a series of tables. His summary of these follows:

1. No water-soluble ferrous compounds have been found in the soils investigated. 2. The lower hydroxides make up the bulk of FeO found (as determined on H_2SO_4 extract). Some ferrous compounds of phosphoric acid are encountered. Small quantities of ferrous compounds of sulfur are found. 3. The highest quantity of FeO is found in the G horizon of the clayey marsh loam soil (1.4 per cent), the smallest in the transition horizons A_2-G and B-G of the podzolic soils with a close glei horizon. 4. The quantity of ferrous iron does not go beyond the limit of 20 per cent of the total iron. 5. The solubility of the FeO compounds varies in the different horizons which are subjected to the gleiing process; it is highest in the upper parts of the glei horizon, in A_2-G and B-G. 6. Not all the forms of FeO compounds in the soil are easily oxidized in the air.

In table 5 Zavalishin presents data on the total constituents of the horizons of a number of glei profiles. He makes the following remarks:

Although the data show no sharp fluctuations in the composition of the different horizons, a number of points are worthy of notice. Of all the constituents the Al_2O_3 seems to be the

most mobile. It is clear that A_2 becomes impoverished and B enriched with Al_2O_3, while the least mobility takes place in the glei horizon. The iron does leach slightly from the G horizon, but in these samples it is weakly expressed. In the podzol sandy types the glei horizon occupies a position intermediate between the true podzol horizon and the parent material, suffering, however, a definite loss of iron. It appears that iron goes into solution only either at the outset of the gleiing process or periodically when the horizon becomes saturated with water enriched with organic substances and primarily with carbonic acid. This phenomenon finds its corroboration in the separation of iron formations in the form of bog iron ore at the lower points of the clayey and peat-glei bogs. In these places the ground waters as they come to the surface become enriched with humus and their products of decomposition.

TABLE 5

Total analyses of several glei podzol soils

(After Zavalishin)

SOIL	HORIZON	ON BASIS OF DRY SOIL			ON BASIS OF MINERAL PORTION OF SOIL				
		Hygroscopic water	Chemically combined water	Humus	SiO_2	Fe_2O_3	Al_2O_3	CaO	MgO
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Podzolic loam with a close glei horizon	A_1	4.93	12.69		73.28	3.82	16.85	2.28	1.84
	A_2	1.25	1.19	1.28	77.48	3.40	13.86	1.84	1.52
	B-G	1.29	2.53	0.40	72.18	2.50	18.44	2.62	1.65
	G	1.75	1.47	0.30	75.10	3.15	15.42	2.84	1.50
Podzolic loam with a close glei horizon	A_1	3.56	10.64		74.24	3.05	14.85	2.76	1.94
	A_2	2.02	4.50		77.35	3.08	13.40	1.88	1.46
	B	2.65	2.20	0.42	74.25	5.25	14.27	2.00	1.50
	G	2.30	1.88	0.20	74.50	3.18	16.30	1.92	1.64
Podzolic sandy soil with a close glei horizon	A_2	0.10	....	....	93.42	0.48	6.08	0.30	0.09
	G	0.25	....	....	92.55	0.62	7.25	0.50	0.14
	C	0.30	....	....	89.92	1.20	7.85	0.80	0.25
Weakly podzolic sandy soil with a glei horizon	G	0.20	....	....	91.25	0.70	6.90	0.50	0.13
	C	0.30	....	....	90.01	1.00	7.15	0.60	0.11

The bases leach but slightly from the G horizon, a great deal less than from A_2. In general there is not much loss of the alkaline earths, the Mg being particularly stable.

Thus the total analyses seem to indicate a certain similarity between the glei and podzolized horizons. The decrease of hygroscopic and chemically combined water in the glei horizon points in the same direction. The similarity, however, is slight and chemically the glei horizon resembles more the parent material than the podzol. Fluctuations in the total analyses of the glei horizon are, of course, probable. These depend in a large measure on the movement of the moisture which brings about the gleiing effects, i.e., the rapidity with which the solution which acts on the soil changes. It is therefore clear that sandy loam glei horizons, because of the aforementioned, will undergo more change than clay horizons. The process of removing substances in the gleiing reactions is a secondary phenomenon. The primary phenomenon—the change from the higher to the lower states of oxidation—finds a higher expression in clays

than in sands. It is well to remember that other soil horizons, even the A_3, might be subject to the gleiing process with the change in the level of the water table. A chemical analysis of such a horizon may lead to wrong interpretations.

OXYGEN AS A FACTOR IN THE PROCESS OF GLEI FORMATION

Tamm (4) investigated the oxygen tension of the ground waters in a number of soils which are affected by a high water table. His highly interesting results have a bearing on the problem of glei formation. In table 6, compiled from Tamm's data, a number of points are brought out.

TABLE 6

Oxygen content of ground waters in soils related to the marsh or bog type of soil formation

(Compiled from Tamm's data)

SOURCE OF SAMPLE	DEPTH	OXYGEN PER LITER OF WATER	SOURCE OF SAMPLE	DEPTH	OXYGEN PER LITER OF WATER
	cm.	*cc.*		*cm.*	*cc.*
Open body of water	...	8.05	Edges or fringes of marshes with a thin layer of peat	120	0.00
	...	8.35		120	0.08
Moraine soil in neighborhood of open body of water	245	3.21		55	0.12
	245	3.72		55	0.12
	245	3.80		100	0.34
	...	3.96		100	0.35
Under soils with a deep humus layer with spots of peat and a moss cover	30	1.61	An island of dry soil in a marsh	80	4.39
	30	0.28		120	2.15
	105	2.53		185	0.42
	105	2.78		225	0.00
	30	0.00	Belt of soil bordering the marsh, with a 15–25 cm. peat layer	30	0.32
	30	0.00		40	0.05
	85	3.04		60	0.54
Areas of peat bordering the extensive marshes	63	3.99		100	1.30
	95	4.68		130	2.20
	95	5.82			
	66	0.00			
	85	1.98			
	70	2.21			
	95	2.70			

A comparison of the oxygen content in the ground waters of moraine soils with that of open bodies of water, springs, and streams, shows that the former contain less dissolved oxygen. However, if we compare the dissolved oxygen content of moraine soils with that of soils containing a deep humus layer with spots of peat and a moss cover, we find that the moraine soils are fairly rich in dissolved oxygen. In the peat areas bordering the extensive marshes the ground waters are rich in oxygen, except at one point, at a depth of 66 cm. On the other hand, the ground waters of the fringes of the marsh, having a thin

layer of peat, are free from, or very poor in, oxygen. Similarly the ground waters of the belt of soils bordering the marsh which, however, have a layer of peat 15 to 25 cm. thick, show a very low oxygen content. According to Tamm, the apparent discrepancies are due to the movement of the ground waters. Whenever there is a free movement of the ground waters from the dry areas through the marsh, there is oxygen even under the peat. In the case of stagnant waters the oxygen is utilized as it penetrates the peat, and the ground waters show a low oxygen content. Tamm points out that rain water percolating through peat loses its oxygen, whereas through a normal humus horizon it is carried downward. Zavalishin (7) cites areas of the latter type with poor stands of pine under which the soil has undergone the effects of gleiing.

Tamm also shows that in cases where the ground waters from a marsh, because of topographic and geologic conditions, flow through coarse-textured strata, ortstein formations appear which ultimately clog the underground channel. Apparently the reduced iron compounds in the marsh waters become oxidized, precipitate, and accumulate as "allochtonic" ortstein (according to Tamm's nomenclature).

Zavalishin (7) infers from Tamm's results and his own observations that the

> . . . oxygen determinations of the ground waters may explain the frequent failures of forest stands in certain areas, the formation of ortstein in conjunction with such areas, and the formation of the glei horizons. According to Tamm the water which percolates through a peat horizon, 40 to 50 cm. deep, is free of oxygen and when such a water system meets an underground stream saturated with oxygen we have two water courses unrelated hydrostatically. This might explain why the more level bluish glei horizons free from iron concretions are encountered under the clayey or peat-clayey horizons A_0 and A_1. They are the least penetrable by the oxygen. At any rate no ortstein formations are found under clayey or peat horizons.

Zavalishin sums up the discussion on glei as follows:

> The glei horizon appears in the form of a sandy or clay material of a light-gray or gray color with a bluish, blue, or sky-blue tinge. The color is not uniform; it depends on the intensity of gleiing and on the mechanical composition of the material. Usually the gray-blue background is mottled with large red spots and veins. Usually these spots are associated with the cracks and root paths and they are more frequent in the clay varieties. The spots around the roots may be of two kinds: if the material is not strongly gleied and there is decomposed organic substance in the root path, then there develops a light-gray-bluish glei formation with a reddish band on the outside. If, on the other hand, the gleiing has proceeded very far and the root path is nothing more than a tube through which air passes, then a red ring forms around it on a light-gray-bluish background. When the gleiing is very strong, the parent material is of a homogeneous gray-blue coloration without any spots or veins. Glei horizons, especially sandy, at times resemble podzols; the bluish tinge and the red spots identify it, however, as glei.
>
> Usually the glei horizons are without structure, more or less compact, sticky, smeary, and appear to be more clayey than the adjoining parent material. A suspension of glei formation does not settle out and it is highly dispersed.
>
> The humus content is higher in the upper layer of the glei horizon than in the lower. The solubility of the humus increases with an increase in gleiing. With that, the reducing property of the glei increases and this in turn influences the reducing reactions with respect to the iron compounds.

A GLEI-LIKE PODZOL IN NEW JERSEY

A study has been made on the effect of a high water table in a podzol sandy soil of the Collington series in New Jersey. The profile sampled is in the Freehold area in the soil survey of New Jersey, between the parallels 40°18′ north latitude and between the meridians 74°08′ and 74°10′ longitude. The profile cut was made in a wooded section just about where the road running north and south in the particular sector crosses the Hockhockson Brook.

Topographic and climatic features.—The topography of the section is level, with minor microrelief features. Geologically it belongs to the Coastal Plain Province, which consists of formations of unconsolidated, almost horizontal beds of gravel, sand, sandy clay, and marls, with a slight dip to the coast. The formations are chiefly Tertiary, the upper Miocene. The climate is characterized by the relatively narrow daily and monthly ranges of temperature. The rainfall is rather heavy, averaging about 50 inches annually, with a minimum of about 40 and a maximum of over 70 inches. The mean annual temperature is about 53°F., with an absolute minimum of −11°F., and an absolute maximum a little over 100°F. During the winter the ground freezes occasionally a few inches deep, but these frosts do not last very long and are alternated by mild weather, which quickly thaws out the frozen ground. The humidity is relatively high except during some periods in the fall. An important feature of the rain-fall element of the climate is its relatively even distribution.

The forest vegetation consists of a stand of second growth hardwoods, 20 to 50 years old: red maple, black and white oak, sweet gum, gray birch, and ironwood. Of the more prominent herbaceous vegetation noted are: smilax, carex, cinquefoil, and star flower.

Morphological characteristics.—The morphological features of the profile under consideration are as follows:

A_0: 0 to 2 cm. deep; forest débris of dead leaves and twigs, some slightly decomposed and some still fresh.

A_1: 10 cm. deep; dark gray with a sprinkling of white silica; structureless; sandy texture; charcoal found in upper portion.

A_2: 25 cm. deep; light brown; sandy texture but heavier at the bottom; structure tending towards lamination; poor in organic matter.

B: 15 cm. deep; reddish brown (coffee color); slightly heavier than A_2; iron incrustations found; more distinctly expressed structure in upper portion.

C: A typical glei formation; color grades into a bluish gray; dark iron concretions are scattered through the horizon; tongues of iron formations penetrate into this horizon. Greensand marl is found below a depth of 65 cm., with the water table, at the time of examination, at that point.

Chemical characteristics.—In table 7 data are presented on the chemical composition of the soil.

The outstanding feature of the data is the high acidity of the soil. Both the pH and the unsaturation clearly show that with depth the acidity increases. This is not in accord with the findings of Zavalishin and the other Russian investigators who claim that the G horizon, as a rule, is less acid than the

overlying horizon. Apparently the composition of the ground waters is a factor in determining the reaction of the glei horizon. In this particular case the ground waters from the adjoining areas have proved to be acid, pH 5.2 and even lower.

If it were not for the absence of a morphologically apparent A_2 horizon, this profile could be interpreted chemically as being a genuine podzol. The high SiO_2 content in A_2 and the accumulation of R_2O_3 constituents in B testify to that. There are, however, a few departures from the true chemical features of a podzol. Thus, for instance, the Ca content is not higher in the B than in the A_2 horizon, the *usual* case in podzols. Neither is the pH of the B horizon typical of a true podzol, in which it is usually higher than in the A_2 horizon. And apparently all of this is affected by the gleiing process, which incidentally is also evident from the morphological description of the B horizon; instead of being 40 to 50 cm. thick, as it is in the adjacent normal podzols, the Lakewood soil series, which was described in an earlier publication (3), is only 15 cm. thick. Apparently the gleiing process has acted on the B horizon, and the

TABLE 7

Chemical composition of a glei podzol sandy soil in New Jersey

HORIZON	pH		SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	K_2O	N	BASE EXCHANGE CAPACITY	UNSATURATION (H-IONS)
	Water extract	Neutral salt solution extract	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*m.e.*	*m.e.*
A_1	5.2	4.2	84.79	1.38	2.1	0.264	0.190	0.360	0.173	14.38	13.17
A_2	5.4	4.6	90.69	1.73	3.5	0.216	0.272	0.485	0.0485	6.36	3.67
B	5.1	4.4	83.82	2.55	6.23	0.189	0.282	0.420	0.0745	9.56	6.55
G	4.9	4.6	84.23	3.08	5.15	0.185	0.363	0.545	0.0855	9.92	8.45

slight variations in the chemical composition of the B and G horizons might be ascribed to that. It is of interest to note that the Mg accumulated in the G horizon, a typical attribute of the gleiing process. The high K content is probably due to the glauconite in the greensand marl, the parent material of this profile.

The Fe_2O_3 content is high in the B horizon as well as in the G horizon. One might look upon this accumulation of iron as a result of the combined action of the process of illuviation and gleiing. No ferrous iron could be demonstrated in the B horizon, but the G horizon gave a distinct reaction for ferrous iron immediately after the profile was sampled. Upon exposure to the air and drying the ferrous iron was oxidized.

The high humus content in the B and G horizons, as evidenced by the high N content, indicates a movement of this constituent from the A horizon. It undoubtedly moves in colloidal state by mutual protection with he iront. With the high iron content in these horizons the ortstein formation noted must be classified as an iron-humus ortstein.

The similarity in base-exchange, or rather cation-exchange, capacity in the B and G horizons is proof of the gleiing process extending its influence also on the B horizon, even though this is not apparent morphologically. The increased base-exchange capacity in the B horizon, in comparison with the A_2, is one of the properties of a podzol.

General statement.—The profile under consideration is located at the fringe of a swampy area, and there are indications that the water coming in from a higher level contains appreciable quantities of dissolved oxygen. The ortstein veins, the orterde concretions, and the tongue-like iron formation projections in the G horizon are ample evidence of the high oxygen content in the ground waters which tends to obliterate some of the gleiing effects.

We may thus look upon this soil as a glei-like podzol, a sort of transition to the peat podzol which adjoins this particular area. After all, the process of gleiing and the marsh type of soil formation, of which the peat podzol is one of the subtypes, are closely related.

At the edge of the podzol zone, in the northern hemisphere, where the forest vegetation begins to dwindle, more and more of the glei process is apparent, more and more is the marsh type the outstanding or prevailing type of soil formation. *Thus the glei podzol in the northern countries testifies to the termination of the podzol process of soil formation and the inauguration of the northern marsh or tundra type of soil formation.*

SUMMARY

A review and discussion of the meager work on gleiing, with special reference to the important investigations of Zavalishin, have been given.

The effect of oxygen in the ground waters on the process of gleiing and incidentally also on the state of the iron compounds has been pointed out in the light of Tamm's work.

Analyses of a glei podzol in New Jersey have been presented and interpreted.

REFERENCES

(1) FROSTERUS, B. 1913 Soil formation in clays of humid regions. *Internatl. Mitt. Bodenk.* 3: 99–130.

(2) GEDROIZ, K. K. 1932 Khimicheskii Analiz Pochvui (Chemical Analysis of Soil), ed. 3, Moscow-Leningrad.

(3) JOFFE, J. S., AND WATSON, C. W. 1933 Soil profile studies: V. Mature podzols. *Soil Sci.* 35: 313–331.

(4) TAMM, O. 1925 Ground water movement and stagnation processes explained by oxygen analyses of the ground waters of North Swedish moraines. Meddel. Statens Skogsförsöksanst. [Sweden] 22, no. 1 (from *Internatal. Rev. Sci. and Pract. Agr.* [Rome] 4: 347).

(5) VUISOTZKII, G. N. 1905 Glei. *Pochvovedenie* 7: 291–327.

(6) WITYN, J. 1924 Die Hauptphasen des Podsolbildungsprozesses. Riga.

(7) ZAVALISHIN, A. A. 1928 Some observations on the study of soils with a close glei horizon. Pamyati K. D. Glinka (K. D. Glinka: A memorial), p. 45–90. Leningrad Sel'sko-Khoz. Inst.

BOOK REVIEWS

Kurs Pochvovedeniya (*A Course in Pedology*). By I. V. TYURIN. State Publishing, Moscow-Leningrad, 1933. Pp. 1–312.

In harmony with the general trend of publications in the U. S. S. R., the text-book of Tyurin treats the subject of pedology from the social-economic, or the so-called "economic determinism," standpoint. Of the rôle of soil in human relations, Tyurin states: "Because of this property [fertility] the soil, at a certain state of development of human society, becomes a means of production and an object to which labor is applied for the purpose of exploiting and increasing its fertility." The author does not maintain this viewpoint much beyond the introduction, although he returns to it here and there throughout the book; otherwise he develops the orthodox views of Dokuchaev. The ten chapters are as follows: I. The origin and composition of the mineral portion of the soil; II. Formation of the organic fraction of the soil; III. The process of the mutual activities of soil formation; IV. The soil as a medium for plant growth; V. Soil classification; VI. The soils of the forest regions of the U. S. S. R.; VII. The soils of the forests steppe and chernozem-steppe regions; VIII. The soil of the forestless regions of the U. S. S. R.; IX. The method of studying soils in nature; X. The physical and chemical methods in investigating soils.

This book is designed for students in the schools of forestry, and consequently very little consideration is given to soils outside the forest zone. Thus the gray, brown, and chestnut-brown soils are hardly mentioned; neither are the saline soils taken up in any detail. In six pages the author covers the soils of the dry and desert steppe and of the tundra. The subject of the forest soils, however, is treated in a clear, concise, yet thorough manner, with special attention to the processes involved in soil formation and to the reactions in connection with the base-exchange complex. The pedologist and, even more so, the forester interested in his substratum, the soil, will find this new contribution of great value.

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Kurs Sel'skokhozyaistvennogo Pochvovedeniya (*A Course in Agricultural Pedology*). By A. N. SOKOLOVSKII. State Publishing, Moscow-Leningrad, 1934. Pp. 1–271.

This book is a departure from the general books on pedology, inasmuch as it treats of the fertility elements of the soil; it is not a pedologic, morphologic, or geographic treatise.

The book is divided into eight parts: I. The problems of the science of the

soil and its rôle in socialistic agriculture; II. The origin of soils; III. Soil colloids and their dynamics; IV. The absorption properties of soils; V. The physical properties of the soil; VI. The chemical and biological processes in soils; VII. The origin of the soil types of the U. S. S. R. and their characteristics; VII. Soil fertility.

Throughout the book the author attempts to harmonize the elements of the pure sciences with the philosophical doctrines of the social-economic theories of Marx, Engels, and Lenin, and it is to these theories only that the author cites references. There are no references to soils literature; neither will one find illustrations, maps, or charts pertaining to soils.

The book is written in a very fine and clear style suitable for students who are to be initiated into the science of soils. The subject is treated from the standpoint of the colloidal reactions in the soil with special reference to the circulation of the calcium ion. In this respect the book is more than a text for beginners: the advanced student will find a comprehensive discussion of the physicochemical reactions of soil colloids and of the perplexing problems of soil fertility.

As an addition to the purely pedological treatises of Glinka, Zakharov, Kravkov, and others, this book is to be highly recommended.

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